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MICROTECHNIQUE FOR WINTER BUDS[1]

BY HUGH P. BELL[2] AND VERA FACEY[3]

Abstract

When preparing winter flower-bud material for microscopic examination, unbroken series of sections cannot be obtained by the ordinary methods of dehydrating, imbedding, etc. This is due to the diverse but characteristic structures found in a resting bud. These structures include heavy impervious protective scales, dense mats of hairs between the young leaves, flowers and bracts, and a delicate embryonic tissue at the tip. A continuous series of sections may be obtained by (1) making use of extremely sharp tungsten needles to remove the more minute scales and bracts, especially those between the embryonic flowers; (2) soaking the material for at least two months in 70% alcohol; (3) using *n*-butyl alcohol instead of absolute alcohol and xylol; (4) keeping the material continuously at low pressures; and (5) using an alcoholic stain. Each of these additional steps helps, but all are necessary for completely satisfactory results. A method by which the special tungsten needles may be made is described. The continuous treatment at low pressures is made possible by using a two ounce bottle fitted with a capillary tube and stopcock. The stains which proved most satisfactory were alcoholic solutions of safranin and acid fuchsin.

The proper preparation of certain winter buds for serial sectioning is a problem that presents many difficulties especially if the buds are flower buds. Papers dealing with the histology of the winter buds always mention the fact that imbedding, etc. could not be carried out by the ordinary methods. As considerable work has been done in the Botanical Laboratories at Dalhousie University with buds in their resting condition, and some of the difficulties in technique overcome, it was thought that it would be of assistance to others to record the methods that proved successful.

A brief description of the structural features which render the usual methods ineffective with winter buds should help to make clear why certain procedures are necessary. These special features can be grouped under three headings, (i) the heavy impervious scales that surround the bud; (ii) the dense mat of hairs between the young leaves, flowers and bracts, and (iii) the delicate condition of all the embryonic tissue at the tip. The heavy scales on the outside are usually covered with a wax-like coating and impregnated with a protective substance which is so efficient that they are impervious not only to water but also to any of the liquids ordinarily used in histological technique. In addition, the tissue of these scales is so tough and the cells so thick walled, that they cut with difficulty and the sections will not adhere

[1] *Manuscript received December 21, 1936.*
Contribution from the Department of Biology, Dalhousie University, Halifax, Nova Scotia.

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to the slide. The hairs around the inner leaves, flowers and bracts do not become completely wetted even with such a liquid as ether. As a result, numerous small bubbles of air are retained between these hairs, even after prolonged and frequent use of the air pump. This air may make imbedding and sectioning impossible. The embryonic tissue of the young flowers or of the growing tip is often so delicate that it is macerated by the most dilute solutions of such softening reagents as hydrofluoric acid. Thus, when one wishes to study these delicate tissues, it is not practical to soften the outer scales and hairs by the usual methods. Owing to the combination and close association of these three structural features, modification of the ordinary routine technique and addition to it are necessary before one can obtain properly stained sections in an unbroken series.

Although the ordinary methods described in the textbooks when taken by themselves are quite inadequate, it must be clearly understood that all these ordinary methods are also necessary. For instance, the buds must be opened, the outer scales removed, all collections must be subjected to prolonged treatment under the air pump after both killing and washing. The special precautions outlined in this paper are necessary in addition to all the ordinary methods. The usual technique, however, is so well known and is so well described in the textbooks, that it is not necessary to review it here.

The winter buds for which the methods were worked out were chiefly from the apple, but buds from various species of the Ericaceae were also used. The killing fluid used was 1% chromacetic. Non-aqueous killing fluids were unsatisfactory, because of their high vapor pressure.

The additional precautions that were found necessary involved the following: dissection with special needles; prolonged soaking in a weak alcohol; the use of *n*-butyl alcohol throughout; a continuous exposure to low pressures; and an alcoholic stain. The subject will be discussed under each of these headings.

Dissection with Special Needles

If the bud is a flower bud of a species such as the apple, the original dissection must include the removal of the small bracts between the embryonic flowers. It is impossible to do this with even the sharpest steel needles. Their points are comparatively so blunt that injury to the delicate tissues and flower primordia is bound to result. Such material as finely drawn out glass, etc. is too brittle to remove these rather tough scales. Dr. G. H. Henderson of the Physics Department at Dalhousie provided us with extremely sharp tungsten needles. With these it was possible to remove the smallest structure without injury to the parts to be examined. Dr. Henderson has very kindly described the method of making these needles, as follows.

"These needles are best made from tungsten wire of about ½ mm. diameter. Cut off about 2 cm. of the wire by careful grinding on an emery wheel. More forcible cutting, as by pliers, usually results in splitting the end of the cut-off wire, rendering it unfit for use. Mount the cut-off piece of tungsten wire

in a metal handle. A convenient way is to insert the wire into a short piece of brass tubing or drilled rod and squeeze or pound the brass so as to grip the wire firmly.

"The needle is sharpened by holding the end of the tungsten wire in a bath of molten sodium nitrite. This is easily done by melting the sodium nitrite carefully in a crucible, by a Bunsen burner. The crucible may be supported on a tripod and the needle by a clamp on a retort stand so that about 2 mm. of the wire dips into the molten nitrite. Small bubbles will be seen to come from the tungsten. After from 5 to 15 minutes the needle will be eaten away to a sharp point. It is saving of time if several needles are so treated at the same time. Caution should be exercised in heating the crucible just sufficiently so that bubbles are given off freely; greater heating may result in ignition.

"The progressive sharpening of the point may be followed from time to time by lifting the needle from the bath and examining it under a magnifying glass. Experience will soon enable one to obtain the degree of sharpness desired. When this is reached the needle should be washed under the tap and allowed to dry; it is then ready for use. It is hardly necessary to add that the finer the point the more carefully must it be used. No finer point should be chosen than is necessary for the work in hand."

Prolonged Soaking in a Weak Alcohol

It was found that after the buds had stood for a considerable time (at least two months) in various liquids, they became infiltered with paraffin much more easily, and cut in a more satisfactory manner. When the processes of dehydrating and imbedding were started and completed in the ordinary way immediately after washing, it was impossible to obtain good serial sections. Even running the material through the dehydrating series very slowly did not help much. This failure to cut and ribbon properly was due to the large number of air bubbles still retained in the mat of fine hairs, and to the tough resistant character of the woody tissue at the base of the bud. The prolonged soaking helped with both these difficulties, for during immersion in a suitable liquid the air gradually disappeared from between the hairs, (presumably it was slowly dissolved by the liquid) and at the same time the tissues of the woody base became sufficiently softened to make cutting possible. Various liquids were used, but the most satisfactory proved to be alcohol of from 50 to 75%. Distilled water or a weaker alcohol resulted in too extensive maceration. The minimum time required for both the removal of the air and the softening of the tissues was two months. A longer period is better, and winter buds kept in 70% alcohol for two years were not injured, and cut perfectly.

Use of n-Butyl Alcohol

Removal of the air by dissolving it is a slow process. If these minute bubbles of gas can be dislodged and floated away by a liquid, much time is saved. This bodily displacement of the small air bubbles is apparently a

surface tension problem. Various liquids with a low surface tension were used. Most of them proved more or less unsatisfactory. The one that did prove satisfactory was *n*-butyl alcohol. The series used was as follows:—

Number of solution	Water, %	Ethyl alcohol, %	*n*-Butyl alcohol, %
1	85	15	0
2	70	30	0
3	50	40	10
4	30	50	20
5	15	50	35
6	5	45	50
7	0	25	70
8	0	0	100

If the tissue was washed in water, it was started in Solution No. 1 and left about 24 hours in each solution. After two washes of pure *n*-butyl alcohol, the material was placed in the oven and paraffin chips added to the *n*-butyl alcohol. From then on, the tissue was carried through the usual number of steps and periods of time to pure paraffin. For preserving the tissue in the *n*-butyl alcohol mixture, Solution No. 4 should be used.

Treatment at Low Pressures

The usual treatments at low pressure during and after killing, washing, dehydrating and imbedding were found to be inadequate. Also there are obvious difficulties associated with the prolonged use of the air pump over a volatile liquid such as an alcohol. The ordinary paraffin baths offered on the market for imbedding "*in vacuo*" were unsatisfactory for various reasons, the chief being that they took care of the imbedding period only. Also the use of any apparatus of large volume in which the air pressure must be reduced to a low value necessitates the continuous operation of an air pump. This is impractical as the volatile dehydrating fluids would evaporate to dryness. In order to subject the tissue continuously to a low pressure, a special but very simple piece of apparatus was devised as follows. An ordinary two ounce specimen bottle is fitted with a rubber cork through which is inserted a glass stopcock. This stopcock must have both inlet and outlet of capillary tubing. If the tubing has a larger bore, it is very difficult to prevent leakage.

Immediately the tissue was washed, it was placed in one of these bottles fitted with the rubber cork and stopcock. Both cork and stopcock were well sealed with a good grade of stopcock grease. The pressure of the air in the bottle was then reduced until the liquid commenced to boil. The stopcock in the tube was then turned off and the air pump disconnected. The tissue was left in this apparatus under low pressure until it was transferred to the next solution. Tests were made with a mercury manometer and it was found that the pressure inside the two ounce bottle had not changed perceptibly even after the apparatus had stood for one or two days. Each time

the liquid was changed the air pump was connected to the bottle and the pressure was reduced as described. In the treatments with the various members of the *n*-butyl alcohol series, the tissue was always kept under low pressure in each solution for at least 24 hours. When the stage for paraffin chips was reached, the whole apparatus was put in the oven. At the pure paraffin stage the same apparatus was used, placed in the oven, and the material kept under a low pressure just as at the other stages. If the bottle was allowed to cool while the air was being exhausted, the paraffin assumed an opaque creamy appearance. This apparently did no harm, and the paraffin became perfectly transparent when melted again in the oven. But better results were obtained if the paraffin was not allowed to solidify. This was accomplished by standing the bottle containing the liquid paraffin in hot water during evacuation. When this was done, the paraffin did not become opaque and creamy and a greater quantity of gas was drawn off. When the tissue was in small pieces, dehydrating fluids and paraffin were saved by standing the bottle and stopcock diagonally during evacuation.

Obviously the pressure inside the bottle could not be reduced below the vapor pressure of the liquid in which the tissue was immersed, and as it was necessary for the tissue to be continuously immersed, there was always a minimum below which the pressure could not be reduced. The procedure outlined above, however, kept the tissue subjected to this minimum pressure from the time it was washed until it was imbedded, the only exceptions being the brief periods during which the liquids were changed. If the material had been standing for some months at atmospheric pressure in either Solution No. 4 of the *n*-butyl alcohol series or in 70% ethyl alcohol, it was found best to run the tissue back to 15% ethyl alcohol and start the low pressure treatment as outlined above from the beginning of the *n*-butyl alcohol series.

Alcoholic Stains

Even after taking all these precautions the sections did not adhere well to the glass if the slides were placed in water. Hence it was found advisable to use alcoholic stains. The most satisfactory were safranin in 50% alcohol for nuclei, and acid fuchsin in 70% alcohol for a general stain.

Each of the five treatments outlined above was tested by itself, and resulted in a decided improvement in the results obtained, but to obtain completely satisfactory results, all five had to be followed.

Details of procedure would differ with different tissues and also with the varying customs of individual investigators, but it might be of assistance to give in outline the method we usually used. It is as follows:

Dissection of buds with special needles.

Dissected buds placed in 1% chrom-acetic killing fluid and immediately subjected to low pressure under the air pump for about 30 min.

Killing fluid changed and buds left in fresh killing fluid for 24 hr.

Washed in running water 24 hr.

Air pump with tissue in water. About 30 min.

For each stage from now on (except when otherwise stated) the tissue must be kept under low pressure in the 2 oz. bottle fitted with capillary tube and stopcock.

15% ethyl alcohol. 24 hr.
30% ethyl alcohol. 24 hr.
50% ethyl alcohol. 24 hr.
70% ethyl alcohol. 24 hr.

After 24 hr. under low pressure, the buds are left in 70% ethyl alcohol and stored in a corked bottle at atmospheric pressure for at least two months. Ethyl alcohol was used up to this point and for storing the tissue, because it is cheaper than *n*-butyl alcohol. Preparatory to imbedding in paraffin, it was found necessary for satisfactory results to return the stored material to water or a weak alcohol, at low pressure, for 24 hr., before running it through the graded alcohols, 24 hr. each, at low pressure, in the following series:—

From storage in 70% ethyl alcohol.
50% ethyl alcohol. 24 hr.
30% ethyl alcohol. 24 hr.
Solution No. 1 *n*-butyl alcohol series. 24 hr.
Solution No. 2 *n*-butyl alcohol series. 24 hr.
Solution No. 3 *n*-butyl alcohol series. 24 hr.
Solution No. 4 *n*-butyl alcohol series. 24 hr.
Solution No. 5 *n*-butyl alcohol series. 24 hr.
Solution No. 6 *n*-butyl alcohol series. 24 hr.
Solution No. 7 *n*-butyl alcohol series. 24 hr.
Solution No. 8 *n*-butyl alcohol series. 24 hr. (1st immersion).
Solution No. 8 *n*-butyl alcohol series. 24 hr. (2nd immersion).

Paraffin chips are then added and the whole apparatus placed in the oven and left there for about 3 hr.

Pure paraffin, 1st immersion. Not less than 4 hr.

Pure paraffin, 2nd immersion. Long enough to make a total of 24 hr. in pure paraffin. It is necessary to change the paraffin once to remove the surplus *n*-butyl alcohol.

Imbed buds in paraffin. It is of course necessary to imbed in the open at atmospheric pressure.

Tissue cut in ribbons, mounted on slides and stained in an alcoholic stain.

Acknowledgments

The apple buds used in this work were collected by the staff at the Laboratory of Plant Pathology, Kentville, N.S. The National Research Council of Canada provided a trained technician for three months to help with the routine part of the work. With the help of this assistant it was possible to experiment in technique methods, and arrive at the results outlined above.

RESISTANCE OF WINTER WHEATS TO HESSIAN FLY[1]

By W. R. Foster[2] and C. E. Jeffery[3]

Abstract

Stage of growth at the time of the spring emergence of the Hessian fly appears to account for the differential resistance of varieties of winter wheat at Saanichton, British Columbia. Varieties more advanced in growth appear to be more resistant or freer from infestation than varieties less advanced. There was a positive correlation ($r = +0.84$) between the number of days to maturity and infestation, and a negative correlation ($r = -0.63$) between the height of the wheat and infestation on April 1, about the time the fly emerges.

The varieties of winter wheat grown on the Dominion Experimental Station at Saanichton, B.C. showed culm infestation as follows: (i) Practically free from infestation (0–4%) Dawson's Golden Chaff O. 24, Dawson's Golden Chaff (O.A.C. 61), Kanred × Dawson's Golden Chaff, Kharkov × Dawson's Golden Chaff, Imperial Amber, O.A.C. 104, Red Rock and Triplet; (ii) Moderately infested (30–80%) Crail Fife, Forty-fold, Hybrid 128, Hussar, Oro, Yanward, and Yaroslav; (iii) Heavily infested (85–100%) Albit, Golden Sun, Jenkins × Ridit, Kharkov, Marshal Foch, Martin, Ridit, Sun, Victor, White Odessa and Yeoman.

Nitrate of soda, superphosphate, and a complete fertilizer broadcast or drilled had no significant effect on Hessian fly damage.

Introduction

In 1934 at Saanichton, British Columbia, Hessian fly infested some winter wheat varieties up to 100% in five replicated and distributed plots, while others were left practically free. Owing to such a heavy infestation and also because a number of the varieties of winter wheat have not been previously tested for resistance, it was thought advisable to record the percentage of infestation, number of days to maturity, and yield. An association was indicated between infestation and number of days to maturity, so height and type of growth were noted on April 1, about the time the fly emerges. In 1935 practically no infestation took place in any variety. The results of 1934 were partially substantiated when a light infestation took place in 1936.

Abundant evidence of a differential infestation of wheat varieties has been reported by McColloch and Salmon (2), Rockwood and Reeher (4), Cartwright and Weebe (1), Painter, Salmon and Parker (3), and others.

According to McColloch and Salmon (2) the Hessian fly may and often does, lay as many, or more, eggs on a variety of winter wheat that remains practically free from infestation as on one that becomes heavily infested.

Our observations agree with Rockwood and Reeher (4) in that the Hessian fly infestation of winter wheat in the Pacific Northwest has been limited almost without exception to that occurring in the spring.

Hessian fly investigation is not one of our projects and we do not propose to carry the work further, but it seems worthwhile to publish the results obtained to date.

[1] *Manuscript received January 4, 1937.*
Contribution from the Plant Pathology Branch, Provincial Department of Agriculture, Saanichton, and Dominion Experimental Station, Saanichton, British Columbia.
[2] *Geneticist and Assistant Plant Pathologist, B.C. Department of Agriculture.*
[3] *Foreman, Dominion Experimental Station, Saanichton, B.C.*

Methods

The winter wheat variety plots used in this experiment consisted of three rod rows replicated five and four times in 1934 and 1936 respectively. The yields were taken on the centre rows, while the percentage of infestation was obtained by examining each culm in the first 10 feet of the right side row.

The dates of seeding the winter wheat plots were October 18, 1932, October 12, 1933, September 26, 1934, and September 26, 1935.

The fertilizer plots consist of five rod rows replicated four times and with check replicated eight times. The yield was taken on the centre row.

Correlations were obtained by using the Pearson's product-deviation method. The formula used was:

$$r = \frac{\Sigma xy}{n\sigma x\sigma y}$$

For measuring the probable variation in r the formula was:

$$\text{P E}_r = 0.6745\,\frac{1 - r^2}{\sqrt{n}}\,.$$

For obtaining the probable errors given in Table I the formula used for each variety was:

$$0.6745 \times \sqrt{\frac{\Sigma x^2}{n\,(n-1)}}\,.$$

Experiments and Results

The percentage of culm infestation caused by the Hessian fly and the number of days to maturity of different varieties of winter wheat grown on the Experimental Station at Saanichton in 1934 are shown in Table I. Culms of the varieties Sun and Yeoman were infested 100% in the five replicated plots, while Egyptian Amber was not infested at all. The relation between the percentage of culms infested and number of days to maturity is shown by the high positive correlation ($r = +0.845 \pm 0.05$).

TABLE I

PERCENTAGE OF CULMS INFESTED AND NUMBER OF DAYS TO MATURITY OF DIFFERENT VARIETIES OF WINTER WHEAT AT SAANICHTON IN 1934

Variety	Culms infested, %	Days to maturity
Albit	97.1 ± 0.9	287
Berkeley Rock	11.9 ± 1.4	271
Crail Fife	66.7 ± 3.0	270
Dawson's Golden Chaff O. 24	0.2 ± 0.1	267
Dawson's Golden Chaff O.A.C. 61	0.2 ± 0.1	270
Egyptian Amber	0.0 ± 0.0	271
Fortyfold	46.6 ± 3.1	270
Golden Sun*	97.2 ± 0.8	289
Hussar	53.1 ± 1.0	277
Hybrid 128	78.8 ± 4.3	285
Imperial Amber	0.2 ± 0.1	267
Jenkins × Ridit	88.9 ± 2.1	284
Kanred × Dawson's Golden Chaff	2.4 ± 0.6	275
Kharkov × Dawson's Golden Chaff	3.8 ± 0.7	273
Kharkov	97.7 ± 0.5	281

TABLE I—*Concluded*

PERCENTAGE OF CULMS INFESTED AND NUMBER OF DAYS TO MATURITY OF DIFFERENT VARIETIES OF WINTER WHEAT AT SAANICHTON IN 1934—*Concluded*

Variety	Culms infested, %	Days to Maturity
Marshal Foch	98.9 ± 0.6	290
Martin	98.7 ± 0.5	284
O.A.C. 104	0.4 ± 0.1	270
Oro	47.1 ± 3.0	275
Red Rock	0.4 ± 0.1	267
Ridit	90.5 ± 2.4	271
Sun	100.0 ± 0.0	290
Triplet	0.4 ± 0.3	272
Victor	99.4 ± 0.3	289
V.I.S. 131*	86.1 ± 3.6	287
White Odessa	90.0 ± 2.6	282
Yanward	32.2 ± 5.2	277
Yaroslav	69.5 ± 2.9	279
Yeoman	100.0 ± 0.0	290

* *Hybrid of Dawson's Golden Chaff × Sun.*

Table II shows the height of varieties of winter wheat on April 1, about the time the flies emerge from the stubble and lay eggs on winter wheat (4), and the percentage of culm infestation by the Hessian fly in 1934 and 1936. Varieties practically free from infestation in 1934 were also free in 1936. The relation between the height of winter wheat varieties on April 1 (about

TABLE II

THE HEIGHT OF WINTER WHEAT VARIETIES ON APRIL 1, AND PERCENTAGE OF CULM INFESTATION BY THE HESSIAN FLY IN 1934 AND 1936 AT SAANICHTON

Variety	Height April 1, in.	1934 Culms infested, %	1936 Culms infested, %
Albit	15.0	97.0	15.7
Baldmin	11.5	97.3	12.8
Berkeley Rock	20.5	11.9	4.7
Crail Fife	16.0	66.7	16.0
Dawson's Golden Chaff O. 24	22.0	0.2	0.0
Dawson's Golden Chaff O.A.C. 61	22.0	0.2	0.2
Egyptian Amber	24.0	0.0	0.0
Fortyfold	15.0	46.6	0.0
Golden Sun	14.0	97.2	11.1
Hussar	14.0	53.1	0.7
Hybrid 128	16.0	78.8	12.5
Imperial Amber	20.5	0.2	0.0
Jenkins × Ridit	10.0	88.9	11.8
Marshal Foch	10.0	98.9	15.5
Martin	23.5	98.7	14.5
O.A.C. 104	15.5	0.4	0.0
Oro	14.0	47.1	13.4
Ridit	15.0	90.5	15.1
Red Rock	23.0	0.4	0.4
Sun	10.5	100.0	15.0
Triplet	13.0	0.4	2.2
Victor	10.0	99.4	11.6
White Odessa	16.0	90.0	12.0
Yaroslav	16.5	69.5	3.2
Yeoman	9.0	100.0	12.7

the time the fly emerges) and the percentage of culm infestation is shown by the negative correlation ($r = -0.628 \pm 0.083$). Both correlations indicate that varieties of winter wheat that have reached a certain stage of growth at the time of the spring emergence escape serious injury.

The yield in bushels per acre of winter wheat varieties from 1933 to 1936 inclusive and the average yield for these four years are shown in Table III. All of the varieties of wheat were practically free from infestation in 1933 and 1935, while in 1934 infestation was very heavy and in 1936 it was light. Table IV shows the average yield of varieties of winter wheat tested from

TABLE III

THE YIELD, IN BUSHELS PER ACRE, OF WINTER WHEAT VARIETIES FROM 1933 TO 1936, INCLUSIVE, AT SAANICHTON

Variety	1933	1934	1935	1936	Average
Albit	24.0	7.4	34.0	31.2	24.0
Baldmin	23.8	19.2	60.1	35.4	34.6
Berkeley Rock	26.9	12.0	49.8	32.0	35.2
Crail Fife	34.3	9.9	46.7	32.3	30.8
Dawson's Golden Chaff O. 24	39.3	16.8	51.5	36.5	36.0
Dawson's Golden Chaff O.A.C. 61	38.1	14.6	63.2	42.3	39.5
Egyptian Amber	32.5	10.5	48.5	32.8	31.1
Fortyfold	37.9	11.2	52.0	41.1	35.5
Golden Sun	41.3	10.4	49.4	27.4	32.1
Hussar	23.2	18.0	47.8	43.2	33.0
Hybrid 128	31.0	11.0	37.5	20.1	24.9
Imperial Amber	25.2	20.3	55.1	42.1	35.7
Jenkins × Ridit	24.8	12.6	28.2	26.6	23.0
Marshal Foch	33.9	9.5	43.5	26.7	28.4
Martin	26.2	15.7	27.0	26.6	23.9
O.A.C. 104	29.4	25.3	49.4	35.2	34.8
Oro	33.2	19.5	28.3	24.0	26.2
Ridit	26.8	15.8	23.4	19.1	21.3
Red Rock	35.9	28.6	48.8	42.1	38.8
Sun	36.4	6.9	40.3	20.5	26.0
Triplet	32.9	27.1	43.3	34.8	35.0
Victor	33.4	10.8	47.6	29.4	30.3
White Odessa	33.5	17.4	38.5	25.5	29.2
Yaroslav	33.1	16.5	41.2	36.1	31.7
Yeoman	30.8	0.0	32.7	26.9	22.6

TABLE IV

THE AVERAGE YIELD OF WINTER WHEAT VARIETIES IN SAANICHTON

Variety	No. of years tested	Average yield, bu. per acre
Dawson's Golden Chaff O. 24	11	42.9
Golden Sun	11	37.8
Imperial Amber	9	40.8
Marshal Foch	11	35.6
O.A.C. 104	11	38.8
Red Rock	11	40.4
Sun	11	33.0
Victor	11	35.2
Yeoman	11	29.6

9 to 11 years. The varieties that are resistant to Hessian fly infestation, Dawson's Golden Chaff, Imperial Amber, O.A.C. 104, and Red Rock, are also the four leading varieties in yield. Their high yield is probably partly due to their resistance to Hessian fly.

The effect of different fertilizers, broadcast and drilled, on the yield of Sun Wheat heavily infested by the Hessian fly in 1934 at Saanichton is shown in Table V. Nitrate of soda, superphosphate and a complete fertilizer did not appear to have any significant effect on Hessian fly damage.

TABLE V

THE EFFECT OF DIFFERENT FERTILIZERS ON THE YIELD OF SUN WHEAT HEAVILY INFESTED BY HESSIAN FLY IN 1934, AT SAANICHTON

Treatment		Yield, bu. per acre
Nitrate of soda, 125 lb. per acre, broadcast		7.73
Nitrate of soda, 125 lb. per acre, drilled		5.63
Superphosphate, 250 lb. per acre, broadcast		6.73
Superphosphate, 250 lb. per acre drilled		6.68
Complete (Nitrate of soda 125 lb. per acre; Superphosphate 250 lb. per acre; Muriate of potash 50 lb. per acre)	Broadcast	5.62
Complete (Nitrate of soda 125 lb. per acre; Superphosphate 250 lb. per acre; Muriate of potash 50 lb. per acre)	Drilled	4.50
Check, no fertilizer		7.83

Discussion of Results

The high positive correlation ($r = +0.84$) between number of days to maturity and infestation, and the negative correlation ($r = -0.63$) between height of plants on April 1 (about the time the Hessian fly begins to emerge and lay its eggs on winter wheat) and infestation, indicate that stage of growth may account for the differential resistance of winter wheat varieties at Saanichton. The 1934 data are particularly attractive owing to the marked difference in culm infestation which ranged from 0–100% among varieties grown in five replicated and distributed plots. Furthermore the 1936 data although only ranging from 0 to about 15% tend to substantiate the 1934 results. Rockwood and Reeher (4) state that, "Fall-sown wheat, if seeded early enough to get a good start before cold weather sets in, usually makes enough growth before the spring emergence of the Hessian fly to escape serious injury". Our results tend to support this statement, and show further that the probable cause for the differential resistance of varieties is the stage of growth at the time of the spring emergence of the Hessian fly. Those varieties most advanced in growth by the time of the spring emergence of the fly appear to be more resistant than those less advanced. Both Rockwood and Reeher's and our results were obtained in the Pacific Northwest. In Kansas, where conditions are quite different, Painter, Salmon and Parker (3) state that, "With the exception of the two characters, grain texture and colour of stem,

fly resistance does not seem to be associated with any other commonly observed character of the wheat plant, such as time of maturity, awn type, glume or kernel colour".

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PHYSIOLOGIC FORMS OF LOOSE SMUT OF WHEAT[1]

By W. F. Hanna[2]

Abstract

Four physiologic forms of loose smut of wheat have been found in Manitoba. Two of these forms were collected in the field, one on Reward and the other on Mindum. The two other forms appeared in the course of artificial inoculations in the greenhouse. The origin of physiologic forms of loose smut of wheat is discussed. It is considered that one of the forms that appeared in the course of the greenhouse inoculations may have resulted from a mutation. Evidence is put forward which indicates that different physiologic forms occur in Eastern and Western Canada. None of the 13 varieties of wheat used in the inoculation experiments proved to be resistant to all physiologic forms. The inoculation of Reward, Marquis, Garnet, and Pentad × Marquis with their own spores for four generations did not result in appreciably increasing the infections on these varieties. It was also shown that the healthy Reward plants that are sometimes present in a population grown from artificially inoculated seed are not resistant to loose smut, but have escaped infection because of faulty inoculation.

Introduction

In 1931 a brief report (6) was made of the occurrence in Manitoba of two physiologic forms of loose smut of wheat, *Ustilago Tritici* (Pers.) Rostr. One of these forms was collected on the durum wheat Mindum, and the other on the common wheat Reward. Prior to the publication of this report, Rodenhiser (10, 11) had found that there were distinct differences in the cultural characters of collections of *U. Tritici* originating in different localities. In all, 14 of these cultural forms were described, but no attempt was made to correlate cultural behavior with differences in pathogenicity. Since the publication of Rodenhiser's work extensive experiments with monosporidial cultures of the smut fungi have been made by a number of workers. In view of the results of these investigations cultures of loose smut of wheat that differ in appearance would not necessarily be expected to exhibit differences in pathogenicity.

The first announcement of the occurrence of physiologic forms of loose smut of wheat appears to have been made by Piekenbrock (8). By inoculating a number of varieties of wheat with several collections of spores, he was able to identify two physiologic forms. In wheat crosses made by him, resistance to loose smut was found to be inherited recessively. Piekenbrock's work was continued by Grevel (4) who studied 19 collections of loose smut from Germany and 29 from foreign countries. This material yielded four physiologic forms, three of which were present in the German collections, while the fourth form originated from a collection of loose smut sent from Turkey.

The results of inoculation experiments published by Tapke (14) in 1929 suggested the probable existence of physiologic forms of loose smut of wheat in the United States. A further contribution to the subject was made by

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Ruttle (13) who noted a striking difference between the pathogenicity of spores collected on Reward wheat in Manitoba and those collected on Honor wheat in the state of New York. Recently, Radulescu (9) made a study of physiologic forms of loose smut in Rumania. The winter wheats in that country were apparently attacked by but one physiologic form, but collections from the summer wheats were found to belong to three distinct forms. These forms corresponded with Forms 2, 3 and 4, previously identified by Grevel.

Experimental

Outline of Investigation

The experiments described in the following pages were begun in 1929. They were undertaken to determine (i) the pathogenicity of collections of loose smut from certain varieties of wheat grown in Manitoba; and (ii) the extent to which the pathogenicity of a loose-smut collection can be modified by propagating it for several generations on a particular variety of wheat. If, in each original smut collection, there were present only a single physiologic form homozygous for pathogenicity, any subsequent changes in behavior might be regarded as the result of mutation. If, on the other hand, each original collection consisted of a mixture of two or more physiologic forms, the selective effect of a number of wheat varieties might be expected eventually to separate each collection into its component forms. This selective effect would probably manifest itself by a progressive increase in virulence on certain varieties and a corresponding decrease on others.

Methods

With the exception of certain field inoculations, which will be referred to later, the floral inoculations and the growing of the inoculated seed to determine the degree of loose-smut infection were carried out in the greenhouse. This work was commenced in the fall of 1929 and was completed in February, 1936. The first inoculations were made with spores collected in the field in 1929 on the varieties Mindum, Kota, and Reward. All subsequent inoculations were made with spores that were the direct descendants of these three collections.

The method of designating the different lots of inoculum, and the relationships among them are shown in Fig. 1. The three original collections made on Mindum, Kota, and Reward were designated respectively Mi, K, and R. The descendants of these three collections were assigned letters and numbers to indicate the varieties through which they had passed. The following abbreviations of varietal names were employed: Mindum (Mi), Kota (K), Reward (R), Marquis (M), Garnet (G), Pentad × Marquis (P × M), Renfrew (Ren), Pentad (P), and Khapli (Kh). Spores labelled (RG_1), for example, were collected from Garnet which had been inoculated with spores from Reward. Similarly, those marked (R M_1 P_1) were collected from Pentad inoculated with spores from Marquis which, in turn, had been inoculated with spores originally collected on Reward.

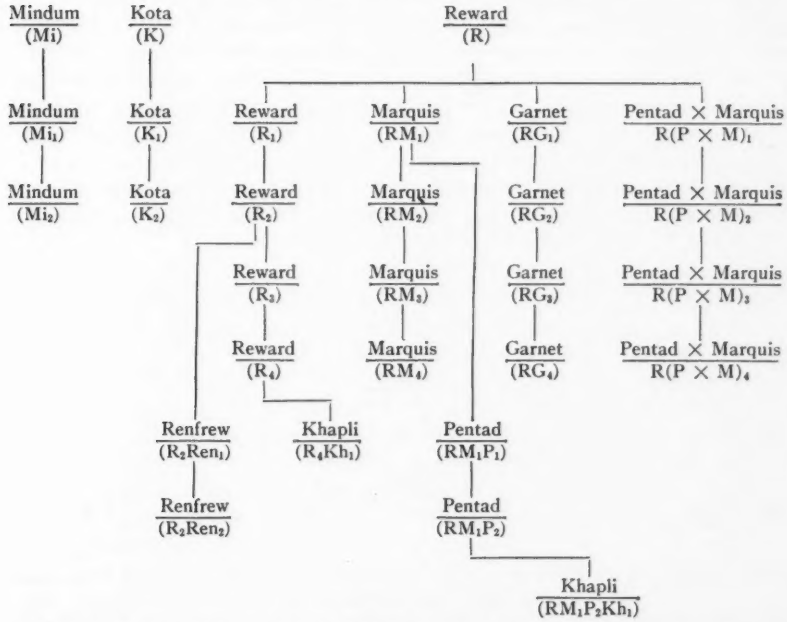

FIG. 1. *Pedigree of loose smut of wheat collections used in inoculation experiments.*

Varieties to be inoculated were grown in pots of soil in the greenhouse. As soon as extrusion of the anthers had commenced the heads were prepared for inoculation by removing with a pair of forceps the small central florets and those at the top and bottom of the spike. The tips of the forceps were then dipped in a vial of spores and the glumes of each floret were forced slightly apart so as to allow spores to fall on the tip of the stigma. The inoculated heads were not bagged, but during the time of seed-setting both temperature and humidity in the greenhouse were kept relatively high.

The percentage infection in the inoculated seed was determined by sowing about one hundred seeds in pots or flats of soil in the greenhouse. When the plants had headed out, the total number of plants and the number of smutted plants from each lot of seed were recorded. The conditions under which different lots of plants were grown were kept as uniform as possible, but variations due to seasonal changes in light intensity could not be prevented.

The literature dealing with the effect of growing conditions on the appearance of smutted heads in wheat grown from infected seed was reviewed recently by the writer (5). The evidence on this subject was somewhat contradictory, but it seemed to indicate that the degree of loose-smut infection is not appreciably influenced by the conditions under which plants are grown. The critical period appears to be at the time of flowering, and Tapke (15) has

shown that if a high humidity is maintained at that time susceptible varieties, when inoculated with viable spores, become heavily infected. A comparison of the results in some of the following tables will show that varying percentages of loose smut were obtained when the same variety was inoculated at different times with the same collection of spores. This variation probably resulted from slight differences in the technique of inoculation, and from lack of uniformity in environmental conditions at the time of inoculation and throughout the period of plant growth. The relative importance which should be attached to each of these factors is at present unknown.

Pathogenicity of Three Collections of Loose Smut

Eleven varieties of wheat were inoculated with loose-smut spores of the three collections, R, K, and Mi, originally made in 1929 from the varieties Reward, Kota, and Mindum. Subsequently the same varieties were re-inoculated with the first, second, and third generations of the R spores, and with the first and second generations of the K and Mi spores. With the exception of the red *durum* variety, Pentad, and the amber *durum*, Mindum, all of the varieties belong to the *vulgare* group of wheats. The percentages of infected plants resulting from these inoculations are given in Table I.

TABLE I

Inoculation of wheat varieties with successive generations of loose-smut spores
(Greenhouse inoculations; Winnipeg)

Variety	Per cent infection									
	R	R_1	R_2	R_3	K	K_1	K_2	Mi	Mi_1	Mi_2
Reward	77	59	86	97	71	87	92	25	1	0
Garnet	91	15	25	37	63	63	88	8	0	0
Marquis	37	30	71	96	25	74	85	3	0	0
Renfrew	0	0	4	2	0	0	0	0	0	0
Marquillo	47	52	86	50	74	96	95	—	0	0
Ceres	72	76	81	89	69	77	100	4	0	0
Preston	4	0	3	12	1	0	0	0	0	0
L. Club	62	2	72	50	53	92	74	0	0	0
Kota	45	32	50	37	65	86	97	—	0	0
Mindum	0	0	0	0	0	0	0	72	77	80
Pentad	0	0	0	0	1	0	0	42	18	2

The results of these inoculations indicate very clearly the existence of two physiologic forms of loose smut. One of these forms, present in the collections from Reward and Kota, infected most of the common wheats, but was unable to infect Mindum, and produced only a slight infection on Pentad in one inoculation. The other form, collected on Mindum, infected this variety heavily, and in the first inoculation produced 42% infection on Pentad, but gave only a light infection on a few of the common wheats. In inoculations with the two subsequent generations of spores, Mi_1 and Mi_2, the high infection on Mindum was maintained, whereas the infection on Pentad diminished, and that on the four common wheats, Reward, Garnet, Marquis, and Ceres, was

reduced to zero. This apparent change in pathogenicity was probably due to progressive purification of the Mi inoculum by repeated passage through the same host. A similar phenomenon has already been noted by Dillon-Weston (2, 3) in his experiments on bunt of wheat.

The only varieties possessing a high degree of resistance to both physiologic forms of loose smut were Renfrew and Preston. These varieties, however, gave low percentages of infection when inoculated with certain collections of spores. The significance of these light infections will be discussed later.

Purification of Loose-smut Collections

Other experiments were made to determine the extent to which a collection of loose-smut spores can be separated into several physiologic forms by repeated passage through particular varieties of wheat. The following varieties of wheat were used in these experiments: Reward, Garnet, Marquis, Pentad × Marquis, Pentad, Mindum, and Khapli. The variety designated as Pentad × Marquis is a stem-rust-resistant hybrid which was produced at the Dominion Rust Research Laboratory. The first inoculations were made with the R strain of spores which had been collected in the field on Reward. This inoculated seed was sown in the greenhouse, and spores were gathered from the smutted plants of Reward, Marquis, Garnet, and Pentad × Marquis to be used for the second inoculation of the seven varieties. These spore collections were labelled respectively R_1, RM_1, RG_1, and $R(P \times M)_1$. Subsequently, all seven varieties were inoculated with the second, third, and fourth generations of spores produced on the four varieties of wheat. The results of all the inoculations are given in Table II.

Inoculation of the varieties listed in Table II with successive generations of their own spores did not result in a progressive increase in infection. Different generations of the same strain of spores, when used to inoculate the variety from which they were collected, sometimes gave widely different percentages of infection. For example, infection on Reward with R_1 spores was only 59%, whereas with R_3 spores it was 97%. This increase in infection was probably not due to the greater virulence of the R_3 spores, although it is possible that this may have contributed in some measure to the increase. Since the inoculations were made at different times and under somewhat different conditions it is probable that the variations in infection should be attributed to these factors rather than to changes in the pathogenicity of the spores.

If the virulence of a strain of spores with respect to a given variety could be enhanced by repeated passage of the spores through that variety it might be expected that the highest mean infection on the variety would be secured by inoculations with several generations of its own spores. By referring to the mean infections resulting from inoculations with the four strains of spores, as given in Table II, it will be seen that the highest infections on Reward and Garnet were obtained by inoculating with spores produced respectively on Reward and Garnet. However, the infection on Reward with Reward spores

TABLE II

INOCULATION OF WHEAT VARIETIES WITH SUCCESSIVE GENERATIONS OF LOOSE-SMUT SPORES

(Greenhouse inoculations; Winnipeg)

Variety	Per cent infection																			
	R	R_1	R_2	R_3	R_4	Mean	RM_1	RM_2	RM_3	RM_4	Mean	RG_1	RG_2	RG_3	RG_4	Mean	$R(P \times M)_1$	$R(P \times M)_2$	$R(P \times M)_3$	$R(P \times M)_4$	Mean
Reward	61	59	86	97	82	77	58	78	71	78	71	64	81	88	62	74	63	88	90	25	67
Garnet	40	15	25	37	92	42	20	77	52	15	41	45	75	74	61	64	17	32	23	7	20
Marquis	49	30	71	96	86	66	66	49	44	32	48	68	75	66	67	69	16	53	53	0	31
Pen. × Marquis	30	43	25	2	33	27	44	30	16	18	27	50	47	28	33	40	14	18	27	11	18
Pentad	0	0	0	0	0	0	1	0	0	0	1	0	0	2	0	1	0	0	0	0	0
Mindum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Khapli	0	0	0	0	5	1	0	0	0	0	0	0	0	0	—	0	0	0	0	0	0

was only slightly heavier than with Garnet spores. On the other hand, the highest infections on Marquis and Pentad × Marquis were secured with spores from Garnet, and not with spores from these two varieties. The relatively high mean infections produced on all four varieties of common wheat by Garnet spores suggests that this strain of inoculum may have been more virulent than the others. Apart from this possible exception, the results in Table II do not lend support to the view that the pathogenicity of loose-smut inoculum can be progressively enhanced by repeated passage through the same host.

Occurrence of New Physiologic Forms

In the discussion of Table I, attention was directed to the light infections that appeared when Renfrew and Preston were inoculated with certain collections of spores. Other light infections occur again in Table II, on Pentad and Khapli. Spores were collected from infected plants of some of these varieties and were used to re-inoculate the varieties from which they had come. In this way it was hoped to isolate and purify any new physiologic forms of loose smut that might have appeared. Renfrew was inoculated with spores gathered from the Renfrew plants on which R_2 spores had produced 4% of smut (Table I). When this inoculated seed was grown, 87% of the plants produced from it were smutted. Spores (R_2Ren_2) from these plants were then used to inoculate 13 varieties of wheat. The results of these inoculations are given in Table III. From the infections obtained on Renfrew, Ceres, and Preston it is apparent that the R_2Ren_2 spores belong to a new physiologic form, quite different from the two forms represented by the R_2 and Mi_2 spores.

Spores from infected plants of Khapli produced by inoculation with R_4 spores (Table II) gave negative results when Khapli was re-inoculated with them. Khapli was also re-inoculated with spores from plants of this variety that had become infected when inoculated with RM_1P_2 spores (Table III). This inoculation gave an infection of only 7% on Khapli, whereas the RM_1P_2 spores had given an infection of 14%.

When Pentad was re-inoculated with spores gathered from the one per cent of infected plants resulting from inoculation with RM_1 spores (Table II), an infection of 24% was obtained. Spores from these plants, designated as RM_1P_2, were then used to inoculate the 13 varieties of wheat listed in Table III.

TABLE III

Reaction of varieties of wheat to physiologic forms of loose smut

(Greenhouse inoculations; Winnipeg)

Variety	Per cent infection			
	R_2	Mi_2	R_2Ren_2	RM_1P_2
Reward	86	0	83	0
Garnet	25	0	80	0
Marquis	71	0	81	0
Renfrew	4	0	66	0
Marquillo	86	0	24	0
Ceres	81	0	0	0
Preston	3	0	88	0
Little Club	72	0	0	0
Kota	50	0	0	0
Pentad × Marquis	25	0	43	0
Pentad	0	2	0	57
Mindum	0	80	0	36
Khapli	0	64	0	14

By referring to this table it will be seen that the infection on Pentad was increased to 57%. Apparently, therefore, the RM_1P_2 spores are distinctly different in pathogenicity from the RM_1 spores from which they originated. They differ also from the Mi_2 spores in the extent to which they infect Pentad, Mindum, and Khapli. In view of these differences the RM_1P_2 strain of inoculum must be regarded as a distinct physiologic form of loose smut.

Selection of Reward for Resistance to Loose Smut

In the inoculation experiments already referred to it will be noted that among even the most susceptible varieties a certain number of plants failed to become infected. The occurrence of these smut-free plants need occasion no surprise when it is considered that ordinary methods of inoculation cannot be relied upon to give perfect infection. It is possible, nevertheless, that a a few of the plants escaped infection, not because of faulty inoculations, but because of their inherent resistance. Standard varieties of wheat are not necessarily pure lines with respect to loose-smut reaction and it is possible that within each variety there are one or more strains differing considerably in smut reaction. If this hypothesis were correct, repeated selection from smut-free plants through several generations might be expected to produce pure lines having greater resistance to loose smut than would be found in a mixed population of the same variety.

With this end in view a number of smut-free plants of Reward, which had appeared in a population grown from artificially inoculated seed, were re-inoculated with spores of the Reward strain of loose smut. The seed from these plants was bulked and, when grown in 1930, it was found that of the plants produced from 141 seeds, 86 or 61% were smutted. Thirty-seven of the 55 plants that had remained smut-free were then chosen for re-inoculation, and one head of each plant was inoculated with Reward spores (R_1 spores) collected from the smutted plants. The inoculated seed from each head was then harvested and sown separately to determine the percentage of infection.

The seed from 16 of the 37 heads produced only smutted plants, so no further selection from these lines was possible. By referring to the first four columns of Table IV it will be seen that seed from the remaining 21 heads produced varying percentages of smutted plants. One head of each of the smut-free plants was then inoculated with spores (R_2) collected from the infected plants, and the seed from each of these heads was grown separately. This procedure was repeated with most of the lines up to the F_3 generation. At this point nearly all of the lines showed from 90 to 100% infection, and it was decided to confine further inoculations to the progeny of Head 16, which had given rather low infection in the F_1 and F_2 generations. This line has been continued to the F_5 generation, which shows 67%.

The data in Table IV give some idea of the extent of variation in infection that may occur when a variety is inoculated at different times and under somewhat different conditions with the same strain of loose smut. As might be expected, the variation in infection was greatest in the first and second

TABLE IV

SELECTION OF REWARD WHEAT IN FIVE GENERATIONS FOR RESISTANCE TO LOOSE SMUT

Head No.	F_1 Generation			F_2 Generation			F_3 Generation			F_4 Generation			F_5 Generation		
	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %	Total plants	Smutted plants	Smut, %
6	11	10	91	8	5	63									
11	1	0	0	16	16	100									
13	3	1	33	14	14	100									
15	17	15	88	9	6	67	18	18	100						
16	19	10	53	78	33	42	259	238	92	59	49	83	39	26	67
17	13	12	92	7	6	86	7	7	100						
18	9	4	44	26	11	42	79	73	92						
20	19	13	68	34	17	50	102	91	89						
21	9	7	78	7	2	29	30	30	100						
22	12	6	50	21	10	48	75	71	95						
23	12	6	50	20	16	80	18	18	100						
25	12	4	33	60	37	62	151	139	92						
28	2	1	50	5	4	80									
29	17	14	82	19	13	68	42	42	100						
31	14	0	0	84	47	56	168	158	94						
32	11	6	55	39	24	62	97	92	95						
33	13	4	31	65	52	80	69	64	93						
34	14	10	71	28	13	46	87	86	99						
35	11	9	82	20	15	75	21	19	91						
36	2	1	50	13	11	85	9	9	100						
37	10	9	90	11	7	64	29	26	90						
	*340	*251	74	584	359	62	1261	1181	94	59	49	83	39	26	67

** Included in this total are the progeny of 16 heads, all of which (109 plants) were smutted.*

generations when most of the lines were composed of only a small number of plants. In the F_3 generation, when greater numbers of plants were dealt with, the infection percentages tend to become more uniform. By the end of the third generation the entire progeny of 24 of the original 37 plants had been destroyed by loose smut, and the progeny of 10 others showed infections of 90% or more. Apparently, therefore, in plants such as wheat, which are normally self-pollinated, the presence among infected plants of a small number of healthy individuals signifies accidental escape from infection rather than inherent resistance.

Occurrence of Different Physiologic Forms in Eastern and Western Canada

The four physiologic forms of loose smut of wheat referred to in Table III all originated in the province of Manitoba. No survey has yet been made of the physiologic forms occurring in other parts of Canada, but there is sufficient indirect evidence to indicate that certain forms occur more frequently in some districts than in others.

In the years 1933, 1934, and 1935 several standard varieties of wheat and a number of stem-rust-resistant hybrids that were being tested for yield and quality in Western Canada were artificially inoculated with loose smut. These wheats were grown in the field at Winnipeg and, during the flowering period, heads were inoculated with spores of the Reward strain of loose smut. The inoculations were made in the same way as the greenhouse inoculations already referred to. The seed harvested from these plants was later sown in pots of soil in the greenhouse to determine the degree of infection.

In 1933, Dr. L. H. Newman, the Dominion Cerealist, arranged to have several of the same varieties of wheat grown at the Dominion Experimental Station, Charlottetown, P.E.I., where they were artificially inoculated with loose-smut spores which had been gathered at that station. Some of this

TABLE V

Reactions of varieties of wheat inoculated with loose smut at Charlottetown, P.E.I., and Winnipeg, Man.

(Field inoculations)

Variety	Per cent infection			
	Charlottetown spores	Winnipeg spores		
	1933	1933	1934	1935
Marquis	12	66	58	78
Reward	42	91	90	79
Ceres	0	71	56	86
Huron		0	1	0
Garnet			68	72
Early Triumph			3	0
(D.C. × H-44) × (D.C.) A.303–1	0	85		
Pentad × Marquis 12–10–3	55	0		

inoculated seed was then sent to the Dominion Rust Research Laboratory, Winnipeg, where it was sown in the field in the spring of 1934. The percentage infection in this seed and in seed of some of the same varieties inoculated at Winnipeg with Reward spores in the years 1933, 1934, and 1935 is given in Table V.

The variety Huron, when inoculated at Winnipeg, proved to be immune in 1933 and 1935, and was only lightly infected in 1934. Unfortunately, this variety was not inoculated at Charlottetown, but a published report (7) indicates that it is susceptible to loose smut in Prince Edward Island. The varieties Ceres, (D.C. × H-44) × (D.C.) A.303–1, and Pentad × Marquis 12–10–3 behaved quite differently towards the two kinds of spores, and their reactions alone show conclusively that the spores used in the inoculations at Winnipeg and Charlottetown represent different physiologic forms.

Discussion

The experiments described in the preceding pages confirm the findings of other investigators that there occur in nature numerous physiologic forms of loose smut of wheat. Different forms, however, tend to predominate in different districts, depending upon the varieties of wheat that are grown. In Prince Edward Island, where Huron wheat is grown, a form attacking this variety has appeared, whereas in Western Canada there are forms attacking Reward, Marquis, Garnet, and Mindum, the varieties most commonly cultivated in the prairie provinces. This tendency of the parasite towards physiologic specialization suggests that new varieties of wheat, unless they possess a high degree of resistance to a number of physiologic forms, may be expected, eventually, to become affected by loose smut.

Although the fact of the occurrence of physiologic forms of loose smut of wheat has been clearly established, comparatively little is known of the manner and frequency of their appearance. Given a mixture of physiologic forms, the host plant may act as a screen in separating them into different parasitic strains. This, however, is a purely mechanical separation and has no relation to the origin of new physiologic forms. In the light of present-day knowledge new forms might be expected to arise by mutation, or by the genetic recombination of two existing forms.

In a recent paper Roemer and Kamlah (12) discussed the manner in which mixtures of physiologic forms of loose smut are reduced to pure lines by repeated passage through the same host. They consider the possibility of the host plant exerting a certain modifying action upon the parasite, resulting in a temporary change in its pathogenicity. According to this hypothesis continued association of host and parasite would result in a more congenial relationship between the two, but would not alter the genetic constitution of the parasite. If the virulence of the parasite could be enhanced in this way, breeding for resistance to loose smut would be ineffectual. It has been well established, however, that resistance to loose smut is inherited in accordance with Mendelian laws. Roemer and Kamlah conclude, therefore, that the

increase in virulence which is sometimes associated with the continued culturing of a collection of loose smut on one host is merely the result of purification of the inoculum by selection.

In the section of the present paper dealing with the data summarized in Table II it was pointed out that the inoculation of Reward, Garnet, Marquis and Pentad × Marquis with their own spores for four generations did not lead to increases in the percentages of smut on these varieties. Failure to increase the infection on Reward, Garnet, and Marquis might be attributed to the fact that these varieties were so susceptible to the parent collection of smut (R) that heavier infections could scarcely be expected. Such an explanation, however, fails to account for the behavior of Pentad × Marquis, which proved to be moderately resistant throughout all of the inoculations. If in the parent collection of inoculum there had been present a few spores of a physiologic form to which Pentad × Marquis was highly susceptible, four generations of selections on this variety would probably have purified and increased it. In the absence of evidence to this effect it may be concluded that the parent collection of spores did not contain such a strain.

It might be expected that continuous cultivation on the same farm of a particular variety of wheat, such as Reward, would result in a few years in the isolation by natural selection of one or more physiologic forms of loose smut highly specialized on that variety. In contrast with the uredospores of the rust fungi which are carried long distances by the wind, with the result that every year new physiologic forms may be introduced into a district, the spores of loose smut of wheat have an effective spread of probably less than a mile. Moreover, uredospores may initiate infection on any part of the plant on which they fall, whereas the spores of loose smut of wheat can only cause floral infection. Consequently, as the distance from the source of inoculum increases the density of loose-smut spores diminishes and the frequency of infection falls off rapidly. It is for this reason that the rogueing of smutted heads from a field as soon as they appear has been recommended as a method of controlling loose smut of wheat. The peculiar manner in which loose smut of wheat causes infection and is carried over from one season to another in the same stock of seed may account for the failure of the inoculation experiments referred to in Table II to effect a separation of the parent collection of spores into different physiologic forms. These spores, which were gathered in a single field of Reward, had probably already been reduced by natural selection to a pure line.

The two physiologic forms, R_2Ren_2 and RM_1P_2 (Table III), appeared during the course of the inoculation experiments. The form designated as R_2Ren_2 attacks Reward heavily and could without difficulty maintain itself on this variety. Consequently, it might have originated as an impurity in the parent collection of R spores. The RM_1P_2 form, however, does not attack Reward and for that reason could scarcely have been present among the R spores originally collected on that variety. The manner of its appearance and its host range suggest that it arose as a mutation from the parent R strain.

Investigations on the inheritance of morphological and physiological characters in *U. Tritici* have been limited because of the difficulty of securing haploid cultures of this fungus. The technique that has been employed with such success in the study of inheritance in the sporidium-producing smuts cannot be adapted to species such as *U. Tritici*, the spores of which on germination produce hyphae and not sporidia. Recently, however, Christensen (1) announced that he had secured haploid cultures of *U. Tritici*. Floral inoculations with a single haploid culture gave negative results, but when the inoculations were made with certain pairs of such cultures a high percentage of kernels became infected. This work has opened up a new and interesting field of research. It will now be possible to obtain lines of *U. Tritici* that are homozygous for pathogenicity, and the relative contributions to the origin of new physiologic forms of this fungus made by hybridization and mutation may be accurately determined.

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THE BEHAVIOR OF PAIRED MONOSPOROUS MYCELIA OF *FOMES ROSEUS* (ALB. & SCHW.) COOKE AND *FOMES SUBROSEUS* (WEIR) OVERH.[1]

By Irene Mounce[2] and Ruth Macrae[2]

Abstract

Fomes roseus and *F. subroseus* are heterothallic and bipolar. With one exception, complete interfertility exists between haploid mycelia derived from different sources. The exception is of particular interest since it shows that two cultures of *F. roseus* from widely separated sources possess one interfertility factor in common. *F. roseus* and *F. subroseus* may be differentiated on the basis of their spore characters. The failure to obtain clamp connections in any of the many pairings of a haploid mycelium of *F. roseus* with a haploid mycelium of *F. subroseus* only serves to emphasize that these two fungi are distinct.

Introduction

Fomes roseus (Alb. and Schw.) Cooke and *F. subroseus* (Weir) Overh. are two fungi which, in young growing specimens, have their context and pore surface pinkish or rose-colored and which may, at times, resemble one another quite closely (Plate I). For many years *F. subroseus* was either confused with *F. roseus* or erroneously named *Trametes carnea* Nees. In 1923 Weir (6) pointed out the distinguishing characters of each fungus and made the new species *Trametes subrosea* to include those usually thinner forms with "darker-colored context, and the conspicuous narrow zonate and radiate fibrillose surface of the pileus" and with narrowly elongated to allantoid spores. In 1933 Overholts (3) transferred this species to the genus *Fomes* and in 1935 (4) again stressed the difference in spore characters: the spores of *F. roseus* (Figs. 1–6) are "elongate-ellipsoid, hyaline, 5–7 × 2.5–3.5μ" while those of *F. subroseus* (Figs. 7–12) are "narrow-cylindric, hyaline, slightly curved, 4–7 × 1–2μ".

Snell, Hutchinson, and Newton (5) in their study of temperature relations of *F. roseus* and *F. subroseus (Trametes subrosea)* obtained such a definite difference in temperature response that they could readily distinguish the two species in culture. These results were in agreement with Weir's conclusions.

Because of the confusion which has existed it seemed worth while to study the behavior of monosporous mycelia of both *Fomes roseus* and *F. subroseus* in order to apply the clamp-connection criterion for the identity of species which Vandendries (7) has stated as follows: "Si les haplontes de deux carpophores sauvages sont toujours et indéfiniment fertiles entre eux ces deux carpophores appartiennent à une même espèce." A preliminary note on this work appeared in the Report of the Dominion Botanist for 1930.

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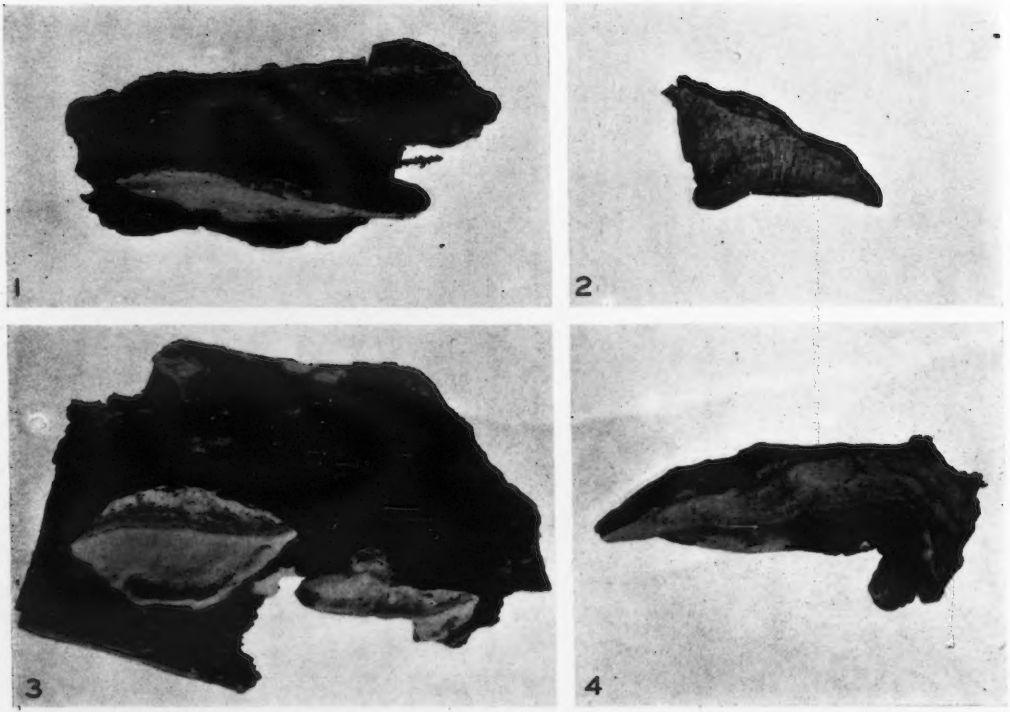


FIG. 1. *Fomes roseus. Sporophore No. 821.* FIG. 2. *F. roseus. Vertical section of Sporophore No. 2355.* FIG. 3. *F. subroseus. Sporophore No. 1658.* FIG. 4. *F. subroseus. Vertical section of Sporophore No. 1639. Figures 1–2 are two-thirds natural size, Figures 3–4 are natural size.*

PLATE I

Isolation of Single Spores

Under the name of *Fomes roseus*, *Trametes subrosea*, or *T. carnea* the cultures listed in Table I were available in the collection at Ottawa.

TABLE I

Culture No.	Host	Locality
368		(From Dr. E. E. Hubert)
376		(From Dr. E. E. Hubert)
693	*Abies balsamea*	Fredericton, N.B.
1000	?	(From Centraalbureau voor Schimmelcultures, Baarn)
1036	*Pseudotsuga taxifolia*	Vancouver, B.C.
1046	*Pseudotsuga taxifolia*	Vancouver Island, B.C.
1299	*Picea rubra*	Cranberry Lake, N.Y.
1300	Wood of coniferous tree	Oswego County, N.Y.
1404	*Picea canadensis*	Timagami, Ont.
1449	*Picea canadensis*	Timagami, Ont.
1639	? *Pseudotsuga taxifolia*	Benton County, Ore.
2355	*Picea canadensis*	Gaspé County, P.Q.
2378	*Pseudotsuga taxifolia*	Point Atkinson, B.C.
2391	Wood of coniferous tree	Keewatin, Ont.
2392	Wood of coniferous tree	Keewatin, Ont.

Sporophores were obtained from cultures grown on prune or malt agar or on small blocks of Douglas fir *(Pseudotsuga taxifolia)* which had been surface sterilized in acetic acid fumes (1) then placed on the slanted surface of prune agar in 250 cc. flasks. Basidiospores were collected on a sterile cover-slip placed beneath a fruit-body, a drop of sterile distilled water was added, and the whole smeared over the surface of lactose gelatine in Petri plates. After germination, isolations were made by cutting out, with a fine needle under the compound microscope, a square of gelatine containing a single spore and placing it in a tube of malt agar. Single spore isolations were made from each of the 15 cultures listed in Table I.

Paired Monosporous Mycelia

From the Same Source

Clamp-connections did not develop on any monosporous mycelium so pairings were made. The results of a series of all possible pairings of 15 haploid mycelia of culture No. 2391 are shown in Table II, and of 15 haploid mycelia of culture No. 2392 in Table III. The plus sign indicates the presence of clamp-connections and the minus sign their absence. Similar series of pairings were made using haploid mycelia from each of the 15 cultures listed above. In every case the haploid mycelia could be divided into two groups. Clamp-connections were formed in every pairing of a member of one group with a member of the other group. The fungi from which the cultures were made are, therefore, heterothallic and bipolar.

TABLE II

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS 15 MONOSPOROUS MYCELIA OF *Fomes roseus* No. 2391

		A							a							
		1	2	3	5	11	12	14	4	6	7	8	9	10	13	15
A	1	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	2	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	3	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	5	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	11	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	14	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
a	4	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	6	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	7	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	10	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	13	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	15	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−

TABLE III

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS 15 MONOSPOROUS MYCELIA OF *Fomes subroseus* No. 2392

		A							a							
		1	3	4	6	8	9	13	14	2	5	7	10	11	12	15
A	1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	3	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	4	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	6	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	8	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	9	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	13	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
	14	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
a	2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	5	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	7	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	10	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	11	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	12	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	15	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−

From Different Sources

Pairings of haploid mycelia from one source with haploid mycelia from each of the other sources show that the 15 cultures can be arranged into two groups as follows:

Group A	Group B	
1404	368	1639
1449	376	2378
2355	693	2392
2391	1000	1036
1299	1300	1046

Haploid mycelia from any culture in Group A are completely interfertile with haploid mycelia from every other culture in that group; that is, they belong to one and the same species. There is one interesting exception to this statement in the behavior of haploid mycelia of Culture No. 2355 when paired with haploid mycelia of Culture No. 2391. It does not, however, alter in any way this general conclusion and will be dealt with separately at the end of the paper. Similarly haploid mycelia from any culture in Group B are completely interfertile with haploid mycelia from every other culture in that group, that is, they, too, belong to one and the same species. Three hundred and eighty-one pairings were made among members of Group A and four hundred and seventy among Group B and Tables IV and V are typical of the results obtained. But though 502 pairings have been made, no

haploid mycelium of Group A has been found that would form clamp-connections when paired with a haploid mycelium of Group B, and Tables VI–VII are typical.

TABLE IV

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. roseus* No. 2391 WITH TWO MONOSPOROUS MYCELIA OF *F. roseus* No. 1449

1449	2391 1	2391 2	2391 3	2391 4	2391 5
3	+	+	+	+	+
7	+	+	+	+	+

TABLE V

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. subroseus* No. 2392 WITH TWO MONOSPOROUS MYCELIA OF *F. subroseus* No. 693

693	2392 1	2392 2	2392 3	2392 4	2392 5
1	+	+	+	+	+
3	+	+	+	+	+

TABLE VI

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. roseus* No. 2391 WITH TWO MONOSPOROUS MYCELIA OF *F. subroseus* No. 693

693	2391 1	2391 2	2391 3	2391 4	2391 5
1	−	−	−	−	−
3	−	−	−	−	−

TABLE VII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *F. subroseus* No. 2392 WITH TWO MONOSPOROUS MYCELIA OF *F. roseus* No. 2391

2391	2392 1	2392 2	2392 3	2392 4	2392 5
1	−	−	−	−	−
4	−	−	−	−	−

The results of all of these pairings are shown graphically in Table VIII in which the plus sign indicates that clamp-connections were formed in every pairing of a monosporous mycelium from the one source with a monosporous mycelium from the other source, and the minus sign that they were absent. Here the members of Group A are labelled *Fomes roseus* and the members of Group B, *Fomes subroseus*.

That the cultures in Group A are *Fomes roseus* and those in Group B are *F. subroseus* may be shown by reference to the shape of the spores obtained from the various cultures that were used. No spores from Cultures 1299, 1036, and 1046 are available at present, but for the rest, spores from Cultures 1404,

TABLE VIII

SUMMARY OF RESULTS OF ALL PAIRINGS SHOWING

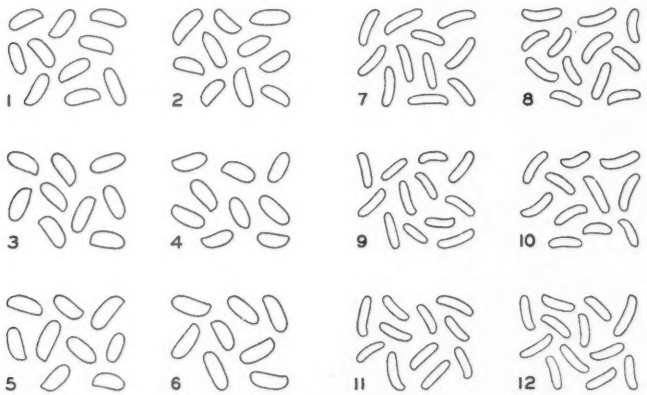
(*a*) Complete interfertility of monosporous mycelia of *F. roseus*

(*b*) Complete interfertility of monosporous mycelia of *F. subroseus*

(*c*) Absence of clamp-connections in every pairing of a monosporous mycelium of *F. roseus* with a monosporous mycelium of *F. subroseus*

		Fomes subroseus									*Fomes roseus*				
		368	*376*	*693*	*1000*	*1036*	*1046*	*1300*	*1639*	*2378*	*2392*	*1299*	*1404*	*1449*	*2355*	*2391*
Fomes subroseus	*368*		+	+	+	+	+	+	+	+	+	−	−	−	−	−
	376	+		+	+	+	+	+	+	+	+	−	−	−	−	−
	693	+	+		+	+	+	+	+	+	+	−	−	−	−	−
	1000	+	+	+		+	+	+	+	+	+	−	−	−	−	−
	1036	+	+	+	+		+	+	+	+	+	−	−	−	−	−
	1046	+	+	+	+	+		+	+	+	+	−	−	−	−	−
	1300	+	+	+	+	+	+		+	+	+	−	−	−	−	−
	1639	+	+	+	+	+	+	+		+	+	−	−	−	−	−
	2378	+	+	+	+	+	+	+	+		+	−	−	−	−	−
	2392	+	+	+	+	+	+	+	+	+		−	−	−	−	−
Fomes roseus	*1299*	−	−	−	−	−	−	−	−	−	−		+	+	+	+
	1404	−	−	−	−	−	−	−	−	−	−	+		+	+	+
	1449	−	−	−	−	−	−	−	−	−	−	+	+		+	+
	2355	−	−	−	−	−	−	−	−	−	−	+	+	+		±
	2391	−	−	−	−	−	−	−	−	−	−	+	+	+	±	

1449, 2355, and 2391 are ellipsoid (Figs. 3–6) while those of Cultures 368, 376, 693, 1000, 1300, 1639, 2378 and 2392 are cylindric and slightly curved (Figs. 9–12). That basidiospores obtained from cultures are identical in color and shape with those of the original specimen has been shown already by the senior author (2). Further proof is given here since Figs. 1, 2, 7 and 8 were drawn from spores produced by wild fruit-bodies while Figs. 3, 4, 9



FIGS. 1–6. *Fomes roseus. Spores elongate ellipsoid.* FIGS. 1 and 3. *Spores from a wild fruit-body No. 2391 and from a culture of that fruit-body respectively.* FIGS. 2 and 4. *Spores from a wild fruit-body No. 2355 and from a culture of that fruit-body respectively.* FIGS. 5 and 6. *Spores from cultures No. 1449 aud No. 1404 respectively.* FIGS. 7–12. *Fomes subroseus. Spores narrow cylindric to slightly curved.* FIGS. 7 and 9. *Spores from a wild fruit-body No. 693 and from a culture of that fruit-body respectively.* FIGS. 8 and 10. *Spores from a wild fruit-body No. 2392 and from a culture of that fruit-body respectively.* FIGS. 11 and 12. *Spores from cultures 1000 and 1300 respectively. (Magnifications × 1025.)*

and 10 were drawn from spores produced in cultures made from these same fruit-bodies. The spores are indistinguishable. Hence the cultures in Group A which, as has been shown by pairing reactions, all belong to one species and all have ellipsoid spores, are cultures of *F. roseus* and those of Group B, which all belong to one species and have cylindric, slightly curved spores, are *F. subroseus*.

The complete interfertility of monosporous mycelia of *F. roseus* from different sources, the complete interfertility of monosporous mycelia of *F. subroseus* from different sources, and the failure to form clamp-connections in any of the 502 pairings of a monosporous mycelium from any of the five different *F. roseus* cultures with a monosporous mycelium from any of 15 different *F. subroseus* cultures is in accordance with the conclusion of Weir, Snell, Overholts, and others that these two fungi, *F. roseus* and *F. subroseus*, are distinct.

Pairings of *F. roseus* No. 2355 with *F. roseus* No. 2391

The unusual behavior of these two strains of *Fomes roseus*, from widely separated districts, may be explained by the hypothesis that they possess one factor in common. Culture No. 2355 was made from a sporophore collected in Gaspé Co., Quebec, on *Picea glauca* by Mr. A. W. McCallum, August 2, 1932. Culture No. 2391 was made from a sporophore collected at Keewatin, Ontario, on wood of a coniferous tree by Mr. M. Timonin, September 24, 1932. Sporophores were obtained in culture, monosporous mycelia were isolated, and pairings made. The results showed that, as usual, each fungus was heterothallic and bipolar, and that monosporous mycelia from these cultures of *F. roseus* were mutually fertile with monosporous mycelia of *F. roseus* from each of the other sources. It was somewhat surprising, therefore, to obtain results such as are shown in Table IX when monosporous mycelia of Culture No. 2355 were paired with monosporous mycelia of Culture No. 2391. To make sure that these results were not due to the age of the monosporous mycelia used, or to the conditions under which they had been kept or to other such factors, subcultures were made from the stock cultures of both No. 2355 and No. 2391. Fresh sporophores were obtained and new monosporous mycelia isolated from them. The results of pairing these mycelia are shown in Table IX. One factor, which we have designated A, was common to both fungi and hence no clamp-connections were formed in one whole group of pairings, where this factor was present in each of the monosporous mycelia being paired.

TABLE IX

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS 16 MONOSPOROUS MYCELIA OF *Fomes roseus* No. 2355 WITH 16 OF *Fomes roseus* No. 2391

		2391B														
		A							a							
		1	4	6	8	9	11	13	26	2	3	5	7	10	12	22	28
A1	1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	4	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	8	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	10	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	13	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	16	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	17	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
2355A																	
a1	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

This unusual case was submitted to Dr. René Vandendries, Rixensart, Belgium, who has very generously given us permission to include his comment.

"Pour ce qui concerne le cas spécial que vous signalez, de *Fomes roseus*, une seule interprétation me paraît possible: il est certain, et votre tableau

l'indique, que les deux souches étrangères l'une à l'autre 2391 et 2355 ont le même facteur A. Pareil fait a été déjà signalé deux fois:

"Par *Hermann Brunswick* sur *Coprinus comatus*. Untersuchungen ueber die Geschlechts—und Kernverhältnisse bei der Hymenomyzeten—gattung Coprinus. (Botanische Abhandlungen 1924. Heft 5, page 109.)

"Par *moi-même* chez *Coprinus radians*. (Je mets moi-même en doute aujourd'hui, s'il s'agit de *C. radians* ou d'une espèce voisine. J'ai eu l'occasion de revoir des cultures de *C. radians* qui n'avaient pas l'aspect des premières et qui étaient homothalles.)

"Du mémoire 'Contribution nouvelle à l'étude de la sexualité des Basidiomycètes,' La Cellule, 3 juin 1924, j'extrais les conclusions générales suivantes:

"3. Les deux sexes qui apparaissent sur un carpophore, différent des deux sexes d'un carpophore étranger.

"4. Une mutation profonde a rendu un haplonte fertile pour tous ses congénères, stérile, au contraire, pour un certain nombre d'haplontes étrangers.

"5. Ce fait nouveau constitue une exception a la loi qui proclame la fécondité constante entre haplontes de carpophores étrangers.

"6. *Le sexe de l'haplonte mutant a pu être identifié avec celui d'un groupe d'individus d'un autre carpophore.*

"Vous vous trouvez devant un cas analogue. Ce qui le rend plus intéressant, c'est que l'existence du facteur A affecte tout un lot d'individus d'une sporée.

"Il diffère de ce que j'ai signalé par le fait que vos individus ne sont probablement pas stériles avec leurs congénères. La différenciation est donc moins prononcée que dans les deux premiers cas signalés.

"Quoi qu'il en soit, j'estime que l'existence de facteurs communs dans des souches étrangères doit être plus répandue que nous le supposons. Cette existence me semble une preuve admirable de la justesse des vues de Kniep, quand il a établi sa théorie des facteurs allélomorphes et de leur origine par mutations.

"Quelle que soit la multiplicité des souches, une origine commune doit fatalement se manifester par des cas d'indentité des facteurs sexuels. Si nous les trouvons rarement, c'est que la Nature est infinie et que nos moyens d'investigation devant cette immensité, est quantité négligeable. Il est miraculeux de pouvoir noter déjà trois cas pareils et c'est bien le vôtre qui est le plus intéressant."

Acknowledgments

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VIRUS STUDIES

III. TOMATO DISEASES[1]

By W. Newton[2] and H. I. Edwards[3]

Abstract

Single virus streak, potato virus X, streak virus X and aucuba mosaic (tobacco virus 6) were found causing diseases of tomatoes in commercial glasshouses in British Columbia during 1936. Single virus streak was the commonest disease although greater losses were caused by streak virus X. Aucuba mosaic was found in one case only, but was highly pathogenic. Potato virus X was present mixed with single virus streak, giving rare cases of mixed virus streak. Tomato mosaic (tobacco virus 1) was not present as a tomato disease.

Single virus streak serum did not give a precipitate when mixed with aucuba antigen, thus indicating that the viruses are distinct. However, a slight precipitate with tobacco virus 1 antigen did indicate distant relationship with this form. Although three strains of single virus streak could be distinguished by symptoms produced on tomatoes when inoculated simultaneously, these strains proved to be serologically identical.

Introduction

A survey of the glasshouses was made in 1936 to ascertain the nature and distribution of the virus diseases that affect tomato production in the coastal regions of British Columbia. Four distinct virus diseases were found and three of these, single virus streak, mixed virus streak and yellow or aucuba mosaic, have been reported as occurring in British Columbia (1). The fourth, streak X, has been described in the second paper of this series (5).

It may be important to distinguish between the diseases that naturally occur in tomatoes and those that can induce disease when transferred from other crops, *e.g.*, tomato mosaic (tobacco virus 1) has not been found affecting tomatoes, but it is quite common as a disease of tobacco in British Columbia. Again, a number of the local potato viruses are significantly pathogenic to tomatoes, but with the exception of potato virus X, no form that naturally occurs in local potatoes has been found in tomatoes.

Experimental

The commercial glasshouses of Vancouver Island and the lower Fraser Valley were inspected at intervals during the tomato production period. When symptoms that suggested virus infection appeared in the crop, leaf samples were taken. These were ground in a small amount of distilled water and transferred to healthy test plants by rubbing the leaf surfaces with a ground glass spatula moistened with the wet leaf pulp. Only four diseases could be sharply differentiated. These are briefly described in Table I.

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TABLE I

THE SYMPTOMS OF TOMATO DISEASES ON SPECIES OF *Solanaceae* AND THE LETHAL TEMPERATURES OF THE INFECTIVE PRINCIPLES

Disease	Lethal temp. 10 min.	Tomato	Tobacco (White Burley)	*N. glutinosa*	*Datura meteloides*	*Datura stramonium*	Petunia	Pepper
Single virus streak	80–85	m,n	L	L	L	L	m	L,m
Mixed virus streak	65–70							
	80–85	M,N	L,m	L,m	L,m	L,m	m	L,m
Streak X	65–70	M,N	Mr	M	m	m	m,n	m
Yellow mosaic	80–85	yM,N	L,yM	L	L	L	yM,n	yM

Explanation of symbols: m = *mottle*, ym = *yellow mottle*, L = *local lesions*, N = *necrosis*. *Capitalization indicates that the symptom is very pronounced.*

Single Virus Streak

The foliage symptoms of single virus streak in tomato ranged from a barely perceptible mottle to a pronounced mottle and crinkle, often accompanied by leaf distortion of the "fern leaf" type. The name of the disease suggests that necrosis of the foliage and stem are characteristic symptoms, but under conditions that approach the ideal for growth of tomatoes, streaked foliage is comparatively rare. Evidence was secured that several strains of single virus streak exist in British Columbia. Three forms were distinguished, characterized by leaf distortion (fern leaf), necrosis and mottle, and mottle only, respectively. However, the strains could only be distinguished by simultaneous inoculations. Under unfavorable growing conditions the least pathogenic form, namely, "mottle", could induce both necrosis and leaf distortion. As will be seen from Table II, the three forms are serologically indistinguishable.

Although single virus streak, tomato mosaic and yellow mosaic have similar lethal temperatures, 80–85° C., which suggests a relationship, nevertheless our serological study has indicated that the relationship is not close. Furthermore, on White Burley tobacco they are readily differentiated. The prominent local lesions induced by single virus streak are rarely followed by a systemic mottle except on very young tobacco seedlings. On the other hand, with tomato mosaic, a systemic mottle is the first symptom to appear. Although local lesions are a primary symptom of yellow mosaic, unlike single virus streak, the local lesions are invariably followed by a pronounced yellow mottle.

Mixed Virus Streak

In agreement with Ainsworth *et al.* (1), mixed virus streak as it occurs in British Columbia was found to be caused by a mixture of single virus streak and potato virus X. Although the infected plants were found at widely different points, one from Vancouver Island and the other from the Lower

Fraser Valley, in both cases the components were identical. The potato virus X component could not be distinguished from the ordinary form isolated from healthy Up-to-date potatoes, on *N. glutinosa*.

Mixed virus streak can readily be distinguished from single virus streak. The development of local lesions on *N. glutinosa* within two days after leaf surface inoculations is a characteristic of both, but in mixed virus streak, the local lesions are followed by a mottle. The local lesions usually appear within two days and the mottle within a week.

Streak X

In host range and in general properties streak X is similar to potato virus X, but on tomatoes it can readily be distinguished from the common strains of X. Streak X induces pronounced necrosis, while ordinary forms of potato virus X induce a faint mottle only. Although streak X is quite a common disease in commercial glasshouses, no case was discovered where streak X was the X component of mixed virus streak.

Yellow or Aucuba Mosaic

The culture of yellow or aucuba mosaic isolated in 1936 appeared to be more virulent or pathogenic on tomatoes than the form forwarded to and reported upon by Ainsworth, Berkeley and Caldwell (1), but otherwise no distinctive properties were found. The symptoms on tomatoes may be confused with the yellow form of tobacco virus 1. The mottles are very similar, but the yellow pigment is more prominent in yellow tobacco mosaic. Apart from the evidence in Table II that the two viruses are serologically distinct, yellow mosaic (tobacco virus 6) produces local lesions followed by a conspicuous yellow mottle on White Burley tobacco, but yellow tobacco virus produces a yellow mottle only.

Serological Tests

Antiserum to single virus streak was prepared by inoculating rabbits with the expressed sap of tomato seedlings infected with a pure strain of this disease. The saps were first purified according to the method of Bawden and Pirie (2) before injecting them into rabbits, and before using them as antigen against the antisera so produced. The rabbit antiserum was further purified by centrifuging out the slight precipitate that forms when purified sap from healthy tomato seedlings is allowed to react with rabbit antiserum. The original and two additional strains of single virus streak were used as antigen, together with tomato mosaic (tobacco virus 1) and yellow or aucuba mosaic (tobacco virus 6). The results are summarized in Table II.

Normal rabbit serum in contact with the five virus antigens developed slight precipitates, but these precipitates were no more abundant than when antiserum was mixed with purified sap from healthy tomatoes. The possible error due to this precipitate was eliminated by pretreatment of the single virus

TABLE II

THE REACTION OF TOMATO VIRUSES AGAINST SINGLE VIRUS STREAK ANTISERUM

Antigen	Antigen dilution with saline	Antiserum dilution with saline	Antigen : antiserum precipitate
Single virus streak, Form 1	1 : 0	1 : 1	+ + + +
	1 : 10	1 : 1	+ + +
	1 : 25	1 : 1	+ +
	1 : 0	1 : 10	?
	1 : 10	1 : 10	+ +
Form 2	1 : 0	1 : 1	+ + + +
	1 : 10	1 : 1	+ + +
	1 : 25	1 : 1	+ +
	1 : 0	1 : 10	+ + + +
	1 : 10	1 : 10	+ +
Form 3	1 : 0	1 : 1	+ + + +
	1 : 10	1 : 1	+ + + +
	1 : 25	1 : 1	+ +
	1 : 0	1 : 10	+ + + +
	1 : 10	1 : 10	+
Yellow mosaic (aucuba) tobacco virus 6	1 : 0	1 : 1	0
	1 : 10	1 : 1	0
	1 : 25	1 : 1	0
	1 : 0	1 : 10	0
	1 : 10	1 : 10	0
Tomato mosaic (tobacco virus 1)	1 : 0	1 : 1	+
	1 : 10	1 : 1	?
	1 : 25	1 : 1	0
	1 : 0	1 : 10	+
		1 : 10	?

streak antiserum with purified tomato sap from healthy tomatoes. The addition of an equal volume of purified sap to the rabbit serum was found to remove completely all non-specific antibodies.

The three strains of single virus streak reacted with a single form of antiserum, hence, in spite of their distinct symptoms on tomatoes they are apparently serologically identical. On the other hand, yellow, or aucuba mosaic failed to react with single virus streak antiserum and hence is serologically distinct. Slight evidence of a relationship between tomato mosaic and single virus streak was suggested by the faint precipitate that formed when tomato mosaic antigen was mixed with single virus streak antiserum.

Discussion

Single virus streak was by far the most common virus disease affecting tomatoes in the glasshouses of British Columbia. At least three strains appear to exist whose characteristic symptom expressions vary from a faint mottle to pronounced leaf distortion and necrosis. The symptoms found in

commercial glasshouses do not serve to identify particular strains owing to the profound alteration of symptoms by environment. A surprising feature of these investigations is that no case of tomato mosaic was found although few glasshouses were entirely free from virus diseases.

From the standpoint of economic importance, streak X is an important disease. Although it occurred less frequently than single virus streak, many cases were found where less than half the plants in a house yielded marketable fruit, owing to the presence of this disease. In spite of its prevalence and the fact that streak X belongs to the potato virus X group, it was never found associated with single virus streak or yellow mosaic in mixed virus streak. When streak X was combined experimentally with single virus streak or with yellow mosaic, both combinations resulted in highly pathogenic diseases. The synthetic disease formed by combining streak X and aucuba or yellow mosaic frequently induced death of tomato plants within ten days. This combination was more pathogenic than any form of "experimental streak" studied in this laboratory.

The form of mixed virus streak that naturally occurs in the commercial glasshouses of British Columbia is quite pathogenic but fortunately its occurrence is comparatively rare. Two cases only were discovered in 1936 although previously other cases were found. Owing to the general prevalence of single virus streak, it was not surprising that mixed virus streak was always a combination of single virus streak and the potato component rather than tomato mosaic and X.

Only one case of yellow or aucuba mosaic was discovered in the glasshouses of British Columbia during the 1936 survey. Although comparatively rare, it was more pathogenic to tomatoes than any other disease that naturally occurs in tomatoes.

The most surprising feature of these investigations was the discovery that single virus streak was serologically distinct from yellow or aucuba mosaic (tobacco virus 6) and tomato mosaic (tobacco virus 1). Most workers have assumed that the viruses that have lethal temperatures between 80 and 85° C. are closely related, partly because their host range is similar and again because no evidence has been presented until recently that they could be distinguished by serological means. Since the completion of these experiments, Chester (4) has shown that tobacco virus 1 and yellow or aucuba mosaic can be differentiated by serological methods. The ordinary technique does not distinguish between the two viruses but if the tobacco virus 1 serum is first absorbed with aucuba antigen and the precipitate is removed, this purified virus 1 serum will again react with virus 1 serum; or conversely, aucuba serum will again react with aucuba antigen after the absorption treatment with tobacco virus 1 antigen. On the other hand Chester (4) found that this residual precipitin reaction did not occur when the serum of masked strain of tobacco virus 1 was absorbed with the normal virus 1 antigen, or conversely, when the normal virus 1 antigen was absorbed with the masked

strain; hence masked tobacco virus may be defined as a true strain of ordinary tobacco virus, but aucuba or yellow mosaic may be considered as a fundamentally distinct virus. Our serological investigation indicates that the British Columbia form of yellow or aucuba mosaic is distinct from single virus streak and that single virus streak is only distantly related to tobacco virus, in spite of the fact that we did not use the absorption technique suggested by Beale (3) and applied by Chester. Unfortunately single virus streak antiserum only was used in our experiments and Chester did not study this virus.

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PRELIMINARY STUDIES OF THE TRANSFER OF FOUR STRAINS OF *DITYLENCHUS DIPSACI* (KÜHN 1858) FILIPJEV 1936[1]

By R. J. Hastings[2] and Wm. Newton[3]

Abstract

The bulb and stem nematode, *Ditylenchus dipsaci*, attacks narcissus, iris, red clover and strawberry in the Pacific Northwest. The isolations from each of these important crops are herein described as strains.

Preliminary studies of the transfer of these strains establish the existence of three strains of *D. dipsaci* in the Pacific Northwest, *viz.:*

(i) Red clover strain; characterized by causing swollen crowns and stunt in red clover seedlings.

(ii) Strawberry strain; characterized by a limited host range, swollen crowns in strawberry seedlings, and entrance into red clover seedlings without visible tissue reactions.

(iii) Narcissus and iris strain; characterized by a wide host range and entrance into clover and strawberry seedlings without visible tissue reactions.

No satisfactory technique of establishing the host range of the biological strains of *D. dipsaci* has been developed. The clamping of glass rings filled with a nematode suspension in moist pulverized peat to the foliage of test plants did not affect the test plants in a constant manner. The examination of seedlings after clarification in a lacto-phenol solution containing acid fuchsin gave more constant results. The seedlings were removed from infested soil shortly after they appeared above ground.

The reports of host specificity of the red clover strain were not confirmed, for the red clover strain entered white clover and alfalfa, hitherto considered resistant. Likewise, the reports of host specificity of the narcissus strain were not supported by our experimental results. The narcissus strain entered red clover and oats, also considered resistant hitherto.

Introduction

Ditylenchus dipsaci, the bulb nematode, is a serious pest on narcissus, iris, red clover and strawberry in the Pacific Northwest. Each of the isolations from these hosts is herein described as a biological strain. It is well known that *D. dipsaci* includes both varieties and biological strains. Steiner and Scott (10) have described the morphological differences of the four varieties, *dipsaci*, *amsinckiae*, *allocotus* and *communis*, but up to the present no morphological differences have been found in biological strains. A biological strain usually derives its name from a host upon which it has been confined for a number of generations, when some degree of specialization is found. Steiner (9) says that "sometimes specialization may reach such a degree that finally even new hosts of the closest taxonomical, physiological, and chemical relationship to the old host are attacked no more or very lightly." However, biological strains exist that may have a wide host range.

Goodey (5) presented evidence to support the existence of biological strains of nematodes in the species *Ascaris lumbricoides*, *Ditylenchus dipsaci* and *Heterodera schachtii*. He referred to the results of different investigators

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that indicated the existence of a polyphagous race of *D. dipsaci* that attacks potatoes and pasture plants; a phlox race that was not highly specialized; a red clover race that would not attack oats, white clover or lucerne; a white clover race that would not attack red clover; and two strains from narcissus that would not attack oats or red clover. He also quoted experiments that suggested that the narcissus and hyacinth nematodes in Holland were biologically different.

Godfrey and Scott (4) recently observed *D. dipsaci* on salsify, parsley, and celery, and by cross inoculations succeeded in inducing infestation of these hosts with nematodes from garlic and parsley but not from alfalfa. They concluded that the salsify, parsley, celery and garlic nematodes were identical, but the alfalfa nematodes were distinct biologically.

The narcissus nematodes in America are apparently quite polyphagous, for Cobb, Steiner and Blanton (3) observed that they entered 29 hosts in the presence of the population host (narcissus). Courtney (1) has transferred the narcissus nematodes to beans, peas, spinach, oats, and vetch; and Hastings, Bosher, and Newton (6) have transferred them to barley, oats, and wheat.

Experimental

Transfer of Narcissus Nematodes to Other Hosts by Leaf Inoculation

The narcissus strain inoculum was secured from narcissus bulbs that had carried infestation for at least four years. The congelations or masses of coiled dormant nematodes from the base of the bulbs were used. The nematodes were placed in direct contact with the leaves by clamping glass rings filled with nematodes and moist peat to the leaves. The nematodes were first activated by suspending them in water before mixing them with the

TABLE I

TRANSFER OF NARCISSUS NEMATODES TO OTHER HOSTS BY LEAF INOCULATIONS

Hosts	No. of leaf sections inoculated	No. of leaf sections with internal nematodes	Hosts	No. of leaf sections inoculated	No. of leaf sections with internal nematodes
Alfalfa	4	0	Orchard grass	12	1
Alsike clover	12	1	Peas	5	0
Awnless brome grass	12	1	Potatoes	13	2
Berseem	2	0	Radish	4	0
Bulbous iris	12	1	Raspberry	5	0
Cabbage	4	0	Red clover	17	3
Carrot	3	0	Reed canary grass	12	1
Common vetch	4	0	Seredella	12	1
Cowpea	39	12	Spinach	29	17
Crested wheat grass	3	0	Sugar beet	15	4
Datura stramonium	15	3	Timothy grass	3	0
Hubam clover	14	2	Tobacco	4	0
Lespedeza	12	1	Tomato	59	34
Lettuce	46	13	White clover	10	2

peat. The inoculated leaf area within the ring was removed at the end of 24 hours, cleared by heating in lacto-phenol and acid fuchsin, and examined under the microscope.

Inconsistent results were obtained, hence the figures are not representative of the susceptibility of the plant involved. The irregular transfers were due to difficulty in maintaining the nematodes in an active state within the confined space of the closed glass ring. Previous studies (7) have shown that the bulb nematodes lose their motility in the absence of fresh air, or in the presence of decaying matter in the medium. After confinement on the leaf in a glass ring filled with moist sand for 24 hr., all the nematodes were inactive when the ring was removed from the leaves, but when the same nematodes were exposed to air in shallow dishes, some recovered their motility. Although moist peat in the rings was found more effective than sand, nevertheless the motility of the nematodes was preserved with difficulty.

Transfer of Narcissus Nematodes to Other Hosts by Soil Inoculation

Steam sterilized soil was inoculated with water suspensions of narcissus nematodes. Various seeds were sown, and a few days after the seedlings appeared above ground they were cut off close to the soil and prepared for microscopic examination by the lacto-phenol method. Owing to difficulty in handling the seedlings after heating in lacto-phenol, they were covered with the solution and heated on the glass slides.

TABLE II

TRANSFER OF NARCISSUS NEMATODES TO OTHER HOSTS BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	6	1	Sainfoin	5	0
Alsike clover	30	3	Siberian millet	15	3
Barley	13	3	Shabdar	35	1
Berseem	10	1	Spinach	6	1
Carrot	5	1	Strawberry	15	4
Cauliflower	9	2	Sweet clover	50	3
Common millet	20	2	Sweet pea	3	0
Japanese millet	20	0	Timothy grass	20	0
Lespedeza	12	0	Tobacco	19	1
Pea	7	2	Tomato	10	3
Reed canary grass	6	0	White clover	5	2
Red clover	17	5			

The nematodes usually entered the seedlings at the crown, in the stem just below it, or in the leaf petioles. Occasionally they entered the leaf blade. Affected red clover seedlings showed a very slight crown enlargement, but the leaf blades and petioles of second and third leaves did not develop per-

ceptible symptoms of infestation. Peas were attacked in the stem and leaf bracts, where white spots appeared, and infestation usually stunted the plants. The appearance of characteristic white spots on barley has been described in a previous publication (6).

The narcissus strain entered 29 hosts in the two experiments.

Transfer of Red Clover Nematodes to Other Hosts by Soil Inoculation

Infested red clover plants were grown in steam sterilized soil. After about six months the nematodes were abundant throughout the soil mass. To create plenty of inoculum the plants, when removed, were ground and mixed with the soil. Various seeds were sown and the seedlings were prepared for microscopic examination by the lacto-phenol method soon after they appeared above ground.

TABLE III

TRANSFER OF RED CLOVER NEMATODES TO OTHER HOSTS BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	15	5	Peas	5	0
Alsike clover	15	3	Red clover	18	5
Awnless brome grass	10	0	Reed canary grass	20	0
Barley	6	2	Siberian millet	13	0
Berseem	21	8	Spinach	4	0
Carrot	7	1	Strawberry	16	3
Common vetch	8	2	Sugar beet	4	2
Hungarian millet	5	0	Sweet clover	15	1
Japanese millet	10	0	Tomato	10	3
Kale	4	0	White clover	17	7
Lespedeza	5	0			

The red clover nematode is evidently polyphagous. Among the crops entered, white clover and alfalfa deserve special mention, since others have reported that the red clover strain does not attack these crops. The infestation of strawberry is of interest, for a natural transfer from red clover to strawberry in Washington State was reported by Courtney (2). Infested red clover seedlings in these experiments developed pathological symptoms. At first a pronounced swelling of the crown appeared and occasionally a bend. As the seedlings grew older, the petioles of the leaves frequently became wrinkled, enlarged and deformed, and the plants were conspicuously stunted. The white spots characteristic of the red clover strain on barley were similar to those induced by the narcissus and iris strains on barley. The nematodes entered most of the seedlings near the crown.

Transfer of Strawberry Nematode to Other Hosts by Soil Inoculation

A single infested strawberry plant was grown in steam sterilized soil. Around the infested plant, strawberry seeds were planted. When the seedlings appeared, their enlarged crowns proved that the soil had become in-

fested. The original plant and the infested seedlings were removed, pulverized, and returned to the soil. Various seeds were sown and the seedlings were examined soon after they appeared above ground.

TABLE IV

TRANSFER OF STRAWBERRY NEMATODES BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	10	0	Seredella	18	0
Alsike clover	34	0	Spinach	7	1
Barley	10	0	Strawberry	6	2
Carrot	20	0	Sugar beet	6	0
Common vetch	8	0	Sweet clover	19	2
Cauliflower	4	1	Tomato	14	0
Peas	4	0	White clover	22	0
Red clover	15	3			

The strawberry strain infested fewer hosts than the other strains under study. Although the strawberry strain entered red clover, no apparent symptom or pathological effects comparable to those produced by the red clover strain were found.

Transfer of Iris Nematodes to Other Hosts by Soil Inoculation

Severely infested Iris "Supreme" bulbs were obtained from local stock known to have been infested for three successive years. The bulbs were crushed in water and the nematode suspensions were washed once in a weak Cheshunt solution, and introduced into autoclaved soil. The seedlings planted therein were examined as previously described, as soon as they appeared above ground.

The host range of the iris nematode appears to be wide. As in the case of the narcissus and strawberry strains the iris nematode entered red clover but did not induce swelling of the crown or other visible pathological symptoms.

TABLE V

TRANSFER OF IRIS NEMATODES TO OTHER HOSTS BY SOIL INOCULATION

Hosts	No. of seedlings examined	No. of seedlings with internal nematodes	Hosts	No. of seedlings examined	No. of seedlings with internal nematodes
Alfalfa	11	1	Spinach	4	0
Alsike clover	30	0	Strawberry	8	0
Barley	27	2	Sugar beet	5	1
Carrot	23	3	Tomato	10	2
Cauliflower	5	1	White clover	16	6
Red clover	15	3			

Discussion

The establishment of biological forms, races or strains of parasitic fungi by differential reactions on distinct hosts has been of great value to agriculture. Breeding for rust- and smut-resistant wheat can now proceed with the reasonable certainty that a new creation will be resistant to the forms present in the country where the new varieties are to be grown. In the case of the bulb nematode, *D. dipsaci*, much work has yet to be done before the identity and relationships of the biological strains are properly known. This investigation establishes the existence of three strains of *D. dipsaci* in the Pacific Northwest, *viz.:*

(1) Red clover, characterized by causing swollen crowns and stunt in red clover seedlings.

(2) Strawberry, characterized by a limited host range and swollen crowns in strawberries; entrance, but absence of symptoms, in red clover seedlings.

(3) Narcissus and iris, characterized by a wide host range; entrance, but absence of symptoms, in red clover and strawberry.

The red clover strain of the Pacific Northwest may be distinct from the form that exists in Great Britain. Goodey (5) reported that the red clover strain would not attack alfalfa and white clover. The form studied by us entered both these hosts.

It is unlikely that the narcissus strain studied by us is distinct from the European form owing to the quantity of European stock that is planted annually in British Columbia. Hodson (8) was unable to induce the narcissus form to attack oats and red clover, but since symptoms rarely appear in oats and have never been observed by us in red clover, the presence of the nematode in these crops may have been missed although it may be easily established by the lacto-phenol acid fuchsin technique.

The results presented herein are essentially preliminary. We have not yet developed a satisfactory technique for establishing the host range of the biological strains of *D. dipsaci*. The clamping of glass rings filled with a nematode suspension in moist pulverized peat to the foliage of test plants did not affect the plants in a constant manner. The examination of seedlings after clarification in lacto-phenol acid fuchsin solution gave more constant results. The seedlings were removed from infested soils shortly after they appeared above ground.

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CONCEPTS AND MECHANISMS OF RESISTANCE IN HELMINTHIC INFECTIONS[1]

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Abstract

The relations that exist between the helminths and their hosts cannot be divided into specific groups. Nevertheless, it is desirable that some classification of these relations, together with clear-cut definition of terms, be widely accepted in order that the subject may be discussed intelligibly. A tentative classification is suggested, which distinguishes between (i) compatibility and incompatibility, which refer only to the host environment as it exists before invasion by the parasite; (ii) resistance, of various kinds and degrees, which refers to the reaction of the environment to the *presence* of the helminth; and (iii) tolerance and intolerance, which refer to the reaction of the environment to the *effects* of the helminth.

The science of immunology deals with the reactions of the body to foreign irritants, organic and inorganic; the organic irritants may include both animal and vegetable parasites, as well as their own products and the products of non-parasitic organisms. Until recently, however, the science has largely been studied as a branch of bacteriology or, to a lesser extent, as a branch of protozoology. Within the past few years, the effects of helminth parasites on the host and the response of the host to these parasites have been under investigation in many laboratories, and numerous papers have been written on the subject. The terminology used, however, has been the terminology of the bacteriologist and, in a number of cases at least, this has resulted in some confusion.

The metazoan parasites are very different in their mode of life from the protista and it does not seem to be possible to interpret their effects without reference to their own actions. The host, on the other hand, is the same, and all the evidence points to an essentially identical mechanism of defense in the host. There is a definite need for a terminology which, describing accurately the relation between host and helminth parasite, will avoid confusion with the already established concepts of the bacteriologist and the protozoologist, and yet will enable the pathologist to reconcile all three points of view.

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The word "helminth," although it has a certain practical utility, is undoubtedly a bad one to use in scientific terminology, and although every definition lays emphasis on the fact that it is a faunistic term, this has seldom been properly appreciated in discussions on immunity. It is necessary to remember that the helminths consist of several completely unrelated phyla, or parts of phyla, which have become adapted through the process of evolution to live in a parasitic environment. To do this, they have adopted many different devices—and their different modes of life are just as varied as those which exist among the vertebrates which they mainly parasitize. It is accordingly very dangerous to draw conclusions of a general nature from observations on a single species. This is especially so when it is realized that we know practically nothing about the physiological processes of the various species. Yet it would seem to be obvious that a helminth that has a purely blood diet, and one that feeds on tissue, bile, mucus or other substances, require different sets of environmental conditions.

The helminth parasite is quite different from the protozoan or bacterial parasite. It enters the host terrain as a young form, grows to adult life, passing as it does so through several stages, and often undertaking an extensive migration through the host tissues; finally it settles down to adult life, mates, and produces eggs or larvae, all of which must ultimately leave the host before they can continue their development. Outside of the host they undergo a certain number of larval changes before they are infective to a new host.

No known helminth can complete its life cycle in the same individual; sooner or later the eggs or larvae must leave the host, and if they are to survive, sooner or later they must gain access to the same or another host. (Even in such apparent exceptions as *Hymenolepis nana*, the eggs normally leave the intestine in the feces to return by the mouth and pass their larval stages in the villi of the small intestine.) This is the fundamental difference between helminths and other parasites.

Helminth parasites must be considered as a collection of individual units each entering the host separately. Bacterial or protozoal parasites are quite different. One enters the body and there it multiplies—sexually or asexually—until an infinitely large number results. They are at best simple creatures, without the complicated morphology, and consequently complicated growth to maturity, found in the metazoan parasites.

The parasitic life of a helminth is dependent on its finding a suitable environment in which it can develop to maturity and in which it can reproduce successfully. This postulates a number of favorable factors.

Factors in Successful Reproduction of Helminths

(a) Successful Admission to the Host

The host must traverse the ground where the free stages of the parasite are found; it must eat suitable foodstuffs in or on which the larval stages occur; it must be exposed to the intermediate host, and so on. Even slight

differences in habits, *e.g.*, such as exist between a sheep and an ox in eating grass, may make all the difference in the parasite gaining admission. The anatomy of the host, including the thickness of the skin, are factors to be considered under this heading.

(b) Suitable Environmental Conditions in the Host

Once inside the host, the parasite must find a suitable habitat—type of mucosa in the intestine, length of intestine, presence of suitable food, and so on. There is a belief, which is not so strongly held now as it once was, that the temperature of the host is of paramount importance, especially in warm-blooded animals. The temperature in edentates however is by no means constant, and yet no wild animal that I have examined has a larger burden of parasitic worms—including forms found in species with more or less constant temperatures—than has the lesser anteater of South America. The influence of hibernation is not well understood nor is the influence of fever. However, clinical experience has shown repeatedly that a high fever will often cause a large proportion of helminths to leave the body, just as an enteritis will cause many intestinal forms to pass out.

(c) Possession of a Suitable Protective Mechanism Against the Normal Metabolic Processes of the Host

When the parasite lies in the alimentary system, it must possess some means of preventing itself being digested or being passed out by peristalsis, etc.

(d) Absence of a Host Reaction that would Interfere with the Normal Metabolism of the Parasite

This factor applies most obviously to parasites that leave the lumen of the alimentary tract at some period of their life, but it may apply to all helminths. If there is any host reaction, the parasites must be able to resist its effects.

These groups of factors are essential for the proper development of the parasite to full and functional maturity. If it finds the environment suitable for its development, we may define the condition as "compatibility"; if it is unsuitable, it is "incompatibility". If the parasite dies at once or is passed out unchanged, the condition is "complete incompatibility". If it develops normally with no unfavorable results to either itself or the host, the condition is "complete compatibility". However, intermediate stages exist and the parasite may grow to some intermediate extent. This intermediate extent may stop short of full maturity; the parasite may never be able to reproduce. On the other hand, it may become mature but not fully so; the adults may be small, the egg output reduced and so on. To enable these to be distinguished from each other, the first is called "partial incompatibility", the second "partial compatibility". None of these conditions has any connection with a host reaction; they merely define the type of environment that the helminth finds in the host.

In complete incompatibility and complete compatibility, no host reaction can occur, but in both partial incompatibility and partial compatibility, a reaction may or may not be present, and this reaction may be directed towards the *effects* of the parasite or towards the *presence* of the parasite.

The reaction of the host to the presence of the parasite is termed "resistance". When the resistance is sufficiently high to prevent the parasite from reproducing (*i.e.*, producing eggs or larvae) it is termed "absolute resistance". This may act at any part of the parasite's life, prior to the actual production of eggs or larvae and it is possible to conceive of it being so pronounced as to destroy the parasite immediately on entry. This theoretical conception, which may be referred to as "virtual resistance", has the same result as complete incompatibility, but differs from it in that it assumes a reaction on the part of the host; complete incompatibility on the other hand, assumes an unsuitability of environment, not necessarily inimicable to the parasite —it may be the absence of something. When the parasite is able to reproduce, in spite of the host reaction, the resistance is termed "partial resistance"; the life of the parasite must in consequence be, to some extent at least, abnormal.

The reaction towards the effect of the parasite is essentially a repair process, whereas resistance involves an active alteration in the parasite's environment. In our present state of knowledge, it seems desirable to keep these two concepts separate. Where the damage done is of a purely mechanical nature for example, it is possible that the reaction is directed entirely towards the repair of this damage and does not affect the worm itself. If the reaction towards the effects is sufficient to counteract and repair them, it is referred to as "tolerance"; if not, then it is called "intolerance". These terms are comparative and depend on a great number of varying factors and they must grade into each other to some extent; intolerance postulates clinical disease—tolerance, sub-clinical disease with no obvious symptoms.

Compatibility, incompatibility and resistance may all be natural or acquired, but an intermediate form of acquired resistance may exist called "premunition." In this form some parasites may develop—either successfully or unsuccessfully—but so long as they remain alive, resistance is offered to further infection; as soon as they die or pass out of the host, the resistance will disappear.

These terms have been defined as though they were hard and fast divisions; it is improbable that they are. Thus too many worms in a completely compatible host would induce a state of incompatibility through lack of food. Tolerance and intolerance obviously grade into each other and both absolute and partial resistance can exist side by side in the same host towards the same species of worm. Premunition may precede and grade into acquired resistance. Moreover, it is at least doubtful whether complete compatibility, incompatibility or resistance are more than theoretical concepts. However, any particular state is true absolutely, not comparatively, for any individual worm.

This scheme accordingly proposes the recognition of five concepts in the relation between host and helminth parasite.

Compatibility, as it refers to the environment as it exists before the invasion by the parasite, will be discussed first. Resistance, as it refers to the reaction of the environment to the presence of the helminth, will be discussed next, and tolerance, as it refers to the reaction of the environment to the effects of the helminth, will be discussed last.

Compatibility

"Compatibility" expresses the idea that the host environment is suitable for the successful development of the parasite, "incompatibility", that the environment is unsuitable. Compatibility does not imply any relationship with virulence as does the term susceptibility in bacteriology; this follows from the fundamentally different life history. In a susceptible host, bacteria will multiply indefinitely; in a compatible host, the number of worms will depend on the number of infective stages gaining admission. There is no evidence at all that the term virulence can be used in connection with helminths in the sense in which it is used in bacteriology.

Obviously, compatibility and incompatibility can be complete or partial. "Complete compatibility" expresses the theoretical idea that the host environment is completely suitable for the development of the parasite. It postulates the absence of any reaction or resistance to the worm as well as the presence of all the necessary environmental factors and it is exactly equivalent to a state of commensalism. Obviously, however, no host can be infinitely compatible as there must be a mechanical limit to the number of parasites harbored, and so this state must grade into incompatibility. Too many parasites may cause a stunting in growth or a reduction in egg output, merely by their own number and without causing any form of reaction in the host.

"Complete incompatibility" expresses the theoretical idea that the host is completely unsuitable for the development of the parasite. It also postulates no host reaction, as the environmental conditions are unsuitable in nature before the advent of the parasite. It is a form of passive protection, but as the word "passive" is used in bacteriology with an entirely different meaning, it seems inadvisable to use it here.

There must exist, however, several intermediate conditions of environment between complete compatibility and complete incompatibility.

The parasite may grow to maturity and lay eggs, but it may be abnormally small in size, or produce abnormally few eggs or live a shorter time than normal. This is defined as "partial compatibility", and expresses the idea that the host environment is more or less suitable. In this group of conditions would be included all cases where there is a reaction on the part of the host to the presence of the mature parasite, as such a reaction must cause some abnormality, no matter how small, in the life of the helminth. Partial compatibility, however, has no necessary connection with any host reaction.

If the parasite grows to some extent, but does not reach maturity, the condition is one of partial incompatibility. The parasite dies or is passed out because conditions are unsuitable, but not so unsuitable as to cause it to die on entry or to be immediately passed out. This state may or may not be associated with any form of host reaction to the presence of the parasite; it has, however, no necessary connection with any form of resistance.

It is quite possible that both complete states are purely theoretical concepts that do not exist in nature and that the partial states are the only ones which occur.

It is probable also that compatibility or incompatibility may be natural or may be acquired by changes in diet, concomitant disease and so on; the so-called "age resistance" may, for example be an acquired incompatibility in mature life and a natural compatibility in youth. The presence or absence of vitamins, minerals and other dietary factors appears in some cases to have much to do with rendering the environment suitable or unsuitable, although as our knowledge advances we may find that these factors are concerned mainly with resistance.

Resistance

If worms fail to become established in a host, it must be because the environment is unsuitable for their establishment, assuming that the larval stages are normal and healthy and can gain admission. This may be the result of incompatibility, which postulates the absence of something essential for the worm's well-being, or resistance to their presence, which postulates the presence of something produced by the host, which is inimical to the parasites. This something may be normally present in the host or it may be produced only on the introduction of the parasite or after some equivalent stimulus; it seems impossible in the present state of knowledge to differentiate sharply between incompatibility and resistance, and it seems difficult to draw a line between natural and acquired resistance. However, such differences must exist.

It is essential, moreover, to select some criterion of resistance against helminths, as "protection" may be shown by the immediate death of the worm or its death at any subsequent period or by its physiological processes being altered so that its life is shortened, its growth stunted, its egg output reduced or its habitat abnormally situated. On the other hand, the parasite may pass out of the body in an active larval stage capable of developing in another animal, or may pass into a quiescent larval stage which also can develop in another animal.

The picture is still further complicated by the fact that the helminths are active animals and may move away from any unfavorable environmental influence, just as can any other animal. It is difficult, therefore, to say that a larva passed in the faeces after entry to the host, has been subjected to any different harmful stimulus from one which was killed *in situ*.

The most perfect form of resistance against any worm would cause its immediate destruction upon entry. Although this might be called "immunity", that term is already used in bacteriology with a different meaning, and until more is known of the parasitic life of helminths and the body response to them, to avoid confusion, the term "virtual resistance" (which is not the same as absolute resistance) is suggested as preferable. This is especially so because complete incompatibility would have an apparently identical result so far as the result is concerned, yet would involve no special body reaction. However, if as the result of a host reaction to its presence, a certain species of worm in a host cannot produce eggs or larvae capable of continuing the cycle, it would be perfect resistance from the biological point of view and this would seem to be our logical concept of absolute resistance.

In practice, while virtual resistance has yet to be proved, it appears that an absolute resistance can and does exist in some cases, although no host is absolutely resistant to all species of helminths. Many of the examples of absolute resistance however, are undoubtedly attributable to external causes, such as climate, opportunity and habits due to race, age, sex, etc.; the animal simply is not exposed to infection. When it is exposed to infection, sooner or later, it acquires a great variety of helminths. Man himself is the best example, because his parasites have been most extensively studied. He has few that are specific to himself alone. Of these, but one genus *(Enterobius)* is shared with other primates and can be considered as a natural species. Practically all the others that occur in human beings are either identical with, or closely related to, forms found in domestic or other animals. It is difficult to escape the conclusion that exposure to infection from habits—especially food habits and close association—is the main factor that decides whether a given parasite occurs in a given host. We have been badly handicapped in our knowledge of this subject by the fact that study has tended to concentrate on the parasites of man and those animals closely associated with them.

As our range of experience extends we are finding that host-parasite specificity is much less common than is generally supposed. Our present information is that it appears highest among the cestodes—but we know even less of their biology than of that of any other worms. It appears lowest among the trematodes. To mention only a few of many possible examples, the common liver fluke *(Fasciola hepatica)* occurs in practically every mammal from man to marsupial; Swales (6, 7) and Krull (4) have recently found that the related *Fascioloides magna* will parasitize a wide variety of hosts; *Brachylaemus* of the opossum will live in dogs, cats and young chickens; the Opisthorchidae and the Heterophyidae have an even wider range of hosts; *Sticorchis* of peccaries will not only live in pigs, but also in edentates and rodents.

Among the nematodes, forms with intermediate hosts tend to show less host specificity than those with a direct life history. Heavy infestations of *Spirocerca* of carnivores, for example, have been found in such animals as donkeys which have been given the opportunity of feeding on dung beetles

(5), while *Dracunculus* occurs in a wide variety of hosts in various parts of the world. Many different species of nematodes with a direct life cycle have been developed experimentally in other animals; thus Daubney in East Africa has recently reported the increasing success with which nematodes of wild ruminants are becoming established in sheep, while the genera *Trichostrongylus* and *Strongyloides*, among others, have a wide variety of natural hosts.

Moreover, many helminths that have difficulty in developing in a "normal" host, can reach maturity after host dietetic modifications, such as avitaminosis, disturbance of mineral balance, starvation, and so on.

While the data are scattered and very incomplete it would seem that physiological similarity is much more important than blood relationship in connection with helminth resistance. Helminths are subject to the same evolutionary laws as are other animals, and sooner or later a mutant arrives which is capable of living in the new environment. The closer the physiological similarity of the new to the old host, the greater will be the opportunity for this.

It follows, accordingly, that a state of partial resistance must exist in many cases, in which the host reaction, while not sufficiently severe to prevent the worm from reaching sexual maturity, yet prevents it from reaching its full size, shortens its life, or only permits a small number of the larvae which gain admission, to develop. A similar result may, of course, follow partial compatibility, and it is not always possible to differentiate those two states, *e.g.*, *Clonorchis* is abnormally small in cases when a large number is present—probably partial resistance—or when in an unusual host such as a rabbit—probably partial compatibility. A similar effect is observed in dogs with a heavy ancylostome infection, when the egg output per worm is considerably reduced.

Natural and Acquired Resistance

Natural resistance is resistance to original infection and is the result of an inherited constitutional peculiarity of the host which interferes with the normal development of the parasite. It is usually a species resistance and cannot usually, in the present state of our knowledge, be separated from incompatibility. It may be absolute or partial.

Natural resistance does not always prevent the entrance of the parasite. Cercarial dermatitis is an example of the failure of the human body to prevent the entry of the larval stages of schistosomes that normally develop in other animals and never develop to maturity in man; so far as our present evidence goes, the human body appears to be naturally absolutely resistant to these worms. In the case of the human "creeping disease" due to dog hookworms, a similar state of affairs exists, except in the case of *A. braziliense* where the natural resistance is partial instead of absolute.

It follows from these examples that, while much natural "resistance" is obviously incompatibility, some is undoubtedly active, especially in helminths, the parasitic life of which is not restricted to the lumen of the alimentary

canal. The fact that an active natural resistance can exist, means that the host response must differ in different species of animals.

"Breed" resistance or incompatibility is closely related to species resistance or incompatibility. Webster has shown that a breed resistance against bacteria exists in mice (as well as a breed susceptibility) and that it can be enhanced by inbreeding. This resistance is to some extent non-specific, as in addition to mouse typhoid, the response against corrosive sublimate was increased. Ackert *et al.* (1) have found some evidence of a breed resistance against Ascaridia in chickens and there is some evidence of a breed susceptibility to *Haemonchus* in sheep.

"Age" resistance is generally taken to mean a "resistance" which increases with the age of the host in the complete absence of infection. There is a considerable amount of evidence to link it with incompatibility, although some of the examples are really acquired resistance. It is generally shown towards parasites of fairly closely related hosts. The young animal has a more generalized type of physiology than the adult and there is a fundamental similarity between the milk-fed young of all mammals; later, when they assume the feeding habits of the adult, the similarity becomes less marked. Moreover, young animals experimentally eat objects that adults refuse and their skin is more easily penetrated by larvae. Accordingly, they should, on first principles, have a larger range of parasites than have the adults of the same species. However, some workers believe that some factor (or factors) is lacking in the young animal, which develops in the adult as the natural consequence of age, in the complete absence of infection (3).

It is impossible at present to differentiate sharply natural resistance from acquired resistance. A resistance evoked against one species may operate to some extent against another. Natural infections—especially those which have not progressed beyond the larval stages—are difficult to eliminate in practice.

Acquired resistance follows and is due to previous infection with helminths; it may be absolute, but is usually partial. Such evidence as is available suggests that the intensity of such a resistance is more or less directly proportional to the amount of tissue damage. Thus, in sheep (8), resistance to *Haemonchus* and *Nematodirus*—blood-sucking nematodes—is more quickly produced and more active than resistance to *Ostertagia*, *Trichostrongylus* and *Cooperia*—non-blood-sucking nematodes. Resistance to larval cestodes developing in somatic positions is relatively great. The few cases of artificially acquired resistance of which we have any knowledge are in this last group. Some workers have also found that they can induce a partial resistance in dogs against *Echinococcus* adults, by the injection of larval cystic material.

Premunition is related to acquired resistance but exists only so long as an original infection with the same species is present; true acquired resistance is subsequent to the infection and survives it. However, premunition may precede true acquired resistance, or may exist simultaneously with the building up of acquired resistance. It is possible that at least some cases of premunition

are due to the accumulation of products of the parasites which make the environment unfavorable to new parasites; such an action might be quite independent of any host reaction and should be classed as acquired partial incompatibility. Undoubtedly many of the examples of the so-called premunition against helminths are based on insufficient data. Some, such as *Hymenolepis nana*, are probably not premunition but true acquired resistance, the cysticercoid stage in the villi having caused the resistance, rather than the adults which have developed from them and are still living in the intestine. Premunition, if it applies at all to infections with helminth parasites, must apply to an infection with more than one individual. The original individual, which induces the resistance, must be able to survive a reaction which forms that enter the host subsequently are unable to survive.

Mechanism of Resistance

Although the mechanisms of active resistance against helminths have been little studied, there is no reason to believe that they differ in essentials from those brought into play against any other foreign invaders. Obviously they must vary from helminth to helminth, depending upon the life processes of the particular parasite under consideration. It is probably true that connective tissue cells form the main body defense and that the response is mainly local rather than general. There is no evidence that any worm produces a toxin in the bacteriological sense, and such noxious substances that do enter the body seem to belong to the class of "foreign proteins," and are the result of the normal metabolism of the parasite.

The presence of eosinophile leucocytes in the blood and other tissues is very common in helminthic infections. There is an increasing amount of evidence to suggest that the function of the eosin-staining granules in these cells is to provide an antidote to foreign proteins, although the cells themselves have phagocytic properties, engulfing and digesting organisms and cells; this property appears to be secondary, however, to their property of rendering foreign proteins innocuous. They are accordingly common in anaphylactic phenomena. A local eosinophilia can be produced only in animals showing eosinophiles in the blood. When these are absent, the eosinotactic substances provoke a considerable accumulation of neutral polynuclear cells. Once this local eosinophilia is established, however, it may persist after the disappearance of the general eosinophilia, and during its establishment it may prevent the appearance of a blood eosinophilia by withdrawing the eosinophiles from circulation.

The general body responses against these dissolved substances are apparently quite strictly analogous to those produced in bacterial infections and there is no need to postulate any special mechanism. As in the case of bacteria, it appears to be affected by defects in diet (including avitaminosis). Ledingham points out that while there is little evidence that nutritional defects disturb the antibody-forming mechanism of the body, there is ample proof that resistance both to spontaneous and induced infection, is profoundly

affected. The precise parts played by particular vitamin deficiencies in producing this lowered resistance are not yet clear. Generally restricted diets may act similarly to diets lacking particular vitamins. The normal excitability of the reticulo-endothelial tissue is greatly reduced by defects of diet which lead to loss of weight. There is a considerable amount of evidence to show that this applies equally to helminths as it does to other foreign stimuli.

Hypersensitivity as the result of helminth infections is a relatively common phenomenon, and allergy is probably a protective mechanism—although it may act harmfully—and it is possible that at least some of the symptoms produced by helminths are the result of hypersensitivity to a relatively harmless parasitic protein. Its normal function is possibly to stimulate a rapid attack on the invading organism or substance and either immobilize or destroy it.

The outstanding reaction against worms however is a local fibrous proliferation. It is the usual inflammatory response which follows the local introduction of any foreign stimulant into the connective tissue of any normal host and serves to isolate the parasite and ultimately to destroy it and repair the tissue damage. Whether the destruction is active or passive is still unknown.

While this reaction is similar in all species of animals it varies considerably in detail from host to host and there is a very urgent need for more detailed studies in comparative pathology. By this is meant a study of the reaction of a variety of hosts to a single cause—such as has recently been carried out by Swales with *Fascioloides magna*. Although known as the "giant American cattle fluke," it is not a normal parasite of cattle. It lives normally in the liver substance of various members of the deer family, especially in elk (or wapiti), coast deer, mule deer and Virginia deer. The lesion in the liver of these animals allows the free exit of ova and thus the life cycle is completed in the presence of suitable snails. However, large Bovidae, including cattle, ranging over the same territory as the deer, ingest the infective cysts on grass, etc., and become infested, the liver appearing dark and irregular in outline and individual lesions appearing, similar in external appearance to common abscesses. The tissue reaction to the presence of the flukes in these animals results in a high eosinophilia and the formation of a hard fibrous cyst from which all means of exit for the eggs are occluded. Thus the parasite is completely enclosed even before it grows to maturity, and its destructive migrations and means of reproduction are stopped. Cattle, accordingly, are absolutely resistant to the presence of this parasite. Sheep, on the other hand, are unable to resist the fluke's migration in the liver tissue and the parasite is, therefore, extremely pathogenic to them and, at least in some cases, death of the host appears to precede the maturation of the parasite. Infected sheep livers appear as obviously diseased organs; discoloration, irregularity of outline and fatty infiltration predominating in the picture. Sheep, like deer, are partially resistant to the presence of the fluke, although their reactions to its effects are different.

Tolerance

Only the effects of the parasite on the host are concerned with disease, and these effects may be due to a variety of causes, negative as well as positive. Apart from the abstraction of foodstuffs and the destruction of other organisms that may be of value to the host, helminths may cause traumatic and other lesions, or produce soluble substances in their excretions or secretions, which are harmful to the host; there is no evidence that any produce toxins in the bacteriological sense. There must be a host reaction to these effects, and if this reaction is sufficient to counteract the effects of the parasite, a state of tolerance may be said to exist; if it does not, then the host is intolerant to the effects of the parasite. It implies no necessary resistance to the *presence* of the parasite although the mechanism of tolerance may be identical with that of resistance. On the other hand it may be quite different. Thus, resistance to the effects of blood-sucking nematodes (such as *Haemonchus*) may be shown by unusual activity on the part of the blood-producing organs, while resistance to their presence may be produced by the connective tissue system. Tolerance will depend upon many factors, external as well as internal, and will vary with individuals, age, diet, intercurrent disease, and so on, and will range from complete tolerance to complete intolerance through an infinite variety of stages. Tolerance can probably be best considered as a repair process and there seems to be no necessity for postulating any specific mechanism different from that used to repair the damage to the host by any other agent. When this process involves a reaction to a dissolved agent, there may be alterations in the serum of the host detectable by test antigens. These do not necessarily play any part in the resistance of the body to the presence of the parasite, any more than do local repair processes necessarily play any part in this resistance.

Tolerance thus used is a comparative term and seems only capable of definition if we assume that it represents the maximum position, *i.e.*, that the body defense against the effects of the parasite is strained to its utmost and that any further increase in effect would break down tolerance. However, any lessening of the effect would not affect the reaction and so tolerance must include all stages from those of an infinitely small nature to those which create the maximum reaction. Beyond this theoretical limit—which must be susceptible of infinite variation due to other causes—we have intolerance, and with intolerance we have the beginning of disease. The greater the intolerance the greater the disease, until at its maximum the host dies. Tolerance on the other hand means the absence of any clinical disease and it may even mean the complete absence of any host reaction to the effects of the parasite because the parasite may have absolutely no effect at all on the host. It expresses the balance of the host-parasite association.

Owing to the delicate adjustment between a host and the species of parasite which is normally found in it, there is less probability of disease than when either host or parasite is unusual. During the process of evolution, the ecology of such a parasite has become so adapted that it does the minimum

amount of damage to the host and so to the chances of continuance of its own species. This adaptation includes not only physiological adaptations of the worm to the parasitic existence in the body, but adaptations of the external larval stages, so that, under normal circumstances, sufficiently few gain admission as not to endanger the host, but not so few as to endanger its own species survival. It is only when these conditions are upset that clinical disease results.

When the parasite is in an unusual host a relatively small number of helminths may cause quite extensive reactions.

Accordingly, intolerance, accompanied by clinical disease is due, as a rule, to large numbers of "usual" parasites or to small numbers of "unusual" parasites in a host. Thus the case of *Fascioloides magna* mentioned above; sheep are very intolerant while the deer tribe are very tolerant to the effects of the parasite.

Relation Between Intermediate Host and Parasite

Many helminths pass part of their larval existence in various animals, called intermediate hosts. In these animals, the parasite cannot (or at least usually does not) reach sexual maturity. There are certain exceptions to this however. In certain species of trematodes for example, eggs may be found in the "cercarial" stage in snails, although there is no evidence that they are normally deposited during this stage. In some cestodes, such as *Taenia solium* in man, the larva may develop to an infective stage in man, as well as in the normal intermediate host, the pig; in this case, however, they cannot develop further and remain as larvae, unless of course, they are eaten by another man.

The host-parasite relation between a larval parasite and an intermediate host would seem to be strictly analogous to that existing between an adult parasite and a definitive host, although the criteria adopted must differ. If for "sexual maturity," the expression "infective stage," is substituted, the concepts of Compatibility-Incompatibility, Resistance, and Tolerance-Intolerance would seem to apply to them also.

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THE PARASITES OF PIGEONS IN CANADA[1]

By M. J. Miller[2]

Abstract

All the helminths found in the domestic pigeon are reviewed and notes on their distribution, location in the host, and pathogenicity are given. Three helminths, *Ascaridia columbae*, *Capillaria columbae* and *Echinostoma paraulum*, have been found in Canadian pigeons and described, *Echinostoma paraulum* being recorded for the first time in North America. The lip characteristics of *Ascaridia columbae*, which until now have been little studied, are described in detail.

The internal parasites of pigeons have been known almost as long as the study of systematic helminthology itself. As far as can be gathered from the available literature, the first mention of a parasite from the domestic pigeon was in 1782 when Goeze recorded *Ascaris teres*. Since then a considerable number of helminths has been described, the three major classes being well represented with members of several families in each class.

A complete list of the helminths that parasitize the pigeon is given at the end of this paper. In the case of those parasites found in other domestic birds as well as in pigeons, the distribution as found in all hosts is given. A few species from the pigeon recorded by earlier authors, and not referred to in the later literature, are indicated by a question mark.

Trematoda

Location in Host

As far as is known all the trematodes of the pigeon are found in the small intestine. The exact position is not constant but usually includes the duodenum and the anterior part of the ileum; however, *Brachylaemus commutatus*, being a caecal dweller in other birds, would most likely be found in the posterior part of the small intestine.

Pathogenicity

By far the most harmful of the trematode parasites in pigeons is *Echinostoma paraulum*. Mönnig (20) states, "Krause and Van Heelsbergen in Europe and Picard in Java have observed deaths in pigeons caused by this parasite. The birds show inappetence, thirst, diarrhoea, lassitude and progressive weakness. At post mortem there is a slight atrophy of the breast muscles and catarrhal enteritis with much mucus, becoming haemorrhagic behind the duodenum."

Echinoparyphium recurvatum causes emaciation and anemia in fowls and would probably produce similar symptoms in pigeons. According to Neveu-Lemaire (24), the presence of *Brachylaemus commutatus* in certain cases causes a hemorrhagic typhlitis. *Hypoderaeum conoideum* is responsible for localized enteritis in ducks and would probably produce the same condition in pigeons. *Apatemon gracilis* has not been reported as being harmful.

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Cestoda

Location in Host

All the tapeworms of pigeons occur in the small intestine. According to Meggitt (19), they are found mostly in the duodenum, *Hymenolepis* nearer the stomach, *Cotugnia* and *Raillietina* farther away. He concludes however that under normal conditions the worms wander from place to place.

Pathogenicity

Generally speaking the cestodes are less harmful to their host than are the other classes of helminths. However, when they become very numerous there is a pronounced effect on health. Meggitt (19), who has carried out considerable work on cestodes of pigeons in Rangoon, writes that extreme emaciation and weakness were the usual accompaniments of a heavy infection. In young birds a heavy infection would probably be fatal.

Nematoda

Location in Host

The nematodes have a wider range in their adaptation to different locations in the host than have the trematodes and cestodes, and they are found from the crop to the posterior part of the alimentary tract. The spirurid worms are usually found in the proventriculus, but may be found in the oesophagus and crop as well. *Acuaria spiralis* is also found in the small intestine. *Ascaridia columbae* is found in the duodenum or just posterior to it. *Eulimdana clava* is found in the connective tissue around the trachea and *E. mazzantii* in the connective tissue of the neck. The remaining two species are found in the small intestine.

Pathogenicity

Of the helminth parasites of pigeons, the nematodes cause the most trouble. The commonest species is *Ascaridia columbae*. Unterberger (1868) was the first to draw attention to the pathogenicity of this parasite. According to Neumann and MacQueen (23) it causes torpidity, loss of appetite, periodic diarrhoea and finally marked wasting, particularly of the pectoral muscles. Death usually occurs at this period. At autopsy, the mucous membrane is distended in patches, more or less large and numerous, engorged with blood, softened or ulcerated and covered with thick mucus. Irwin Smith (14) reports it as being responsible for the death of squabs in New South Wales. Le Roux (17) attributes the death of pigeons in Natal to this parasite. It should be noted however that *A. columbae* is a very common parasite of pigeons, and adult birds, when lightly infected, normally show no symptoms of disease, although when as many as 400 to 500 worms are found in a single bird, as has been reported, they would probably cause grave disorders. The greatest damage is done to young squabs, and among them the highest mortality occurs.

Capillaria columbae is also very common and when present in large numbers is distinctly pathogenic. Neumann (22) quotes Pauly and Zurn as stating that it often causes an intense intestinal catarrh which leads to anemia and

debility. According to Neveu-Lemaire (24) autopsy of an affected bird shows the intestinal mucosa to be grayish, tumefied and covered with striae and petechiae.

Cram (8) reports heavy infections of *Acuaria (Dispharynx) spiralis* as causing severe losses among carrier pigeons of the Signal Corps of the United States Army. Symptoms described by Whitney are droopiness and loss of weight with retention of a ravenous appetite, as well as loss of pigmentation of the iris, probably due to anemia. A post mortem showed great enlargement of the proventriculus, the inner surface being a thick necrotic mass containing large numbers of *A. spiralis*. Microscopically there appeared to be almost complete destruction of the glands and a marked infiltration of the underlying tissue.

Ornithostrongylus quadriradiatus is very often fatal to large numbers of birds. According to Cram (9) 134 worms are the minimum required to cause death. This species was described in 1904 by Stevenson who mentioned it as the cause of death of large numbers of pigeons in Washington. Komarov and Beaudette (16) reporting on three flocks, state that in one, 46 out of 50 birds died; in another, 20 out of 100; and in the third flock of 17 birds, all died because of this parasite. Le Roux (17) reports the death of pigeons in Natal from the same worm. According to Stevenson, when worms are present in large numbers, death is caused by a general disorder of the nutritive function of the host due to bacterial infection of the pierced mucosa and the loss of blood.

In *Tetrameres* spp. a great deal of damage is done by the female parasite which migrates into the wall of the proventriculus causing marked irritation and inflammation. According to Timon-David (29) who studied the histopathology of *T. fissispina* in the pigeon, there is a true digesting away of the glands by the enzyme of the parasite. Neumann and MacQueen (23), quoting Zurn, state that this parasite kills young ducks. It would probably act similarly in pigeons.

Helminths of Canadian Pigeons

In Canada, according to Swales (28), two helminths have been recorded from the domestic pigeon, *viz.*, *Ascaridia columbae* (by Wickware, 1922 and Rayner, 1932) and *Capillaria columbae* (by Rayner, 1932). Rayner's records are both from Quebec.

The bulk of the material examined in the present investigation was supplied by Messrs. Henry Gatehouse and Son, Montreal. It consisted only of the posterior part of the alimentary tract, usually including the stomach, and was received in lots of 60–80 at irregular intervals. As a result the material was starting to decompose and had to be examined rather hurriedly. The remaining pigeons were obtained locally and kept in wire cages in the Institute of Parasitology. They consisted of 22 in all, 11 Homers and 11 Tumblers.

In the Gatehouse material, 232 intestinal tracts were examined and, on the whole, surprisingly few parasites were found. Sixteen echinostomes (*Echinostoma paraulum* Dietz, 1909), 12 larval nematodes and three capillarids were found. The larval nematodes were undoubtedly ascaridias and most likely *A. columbae*. In comparing them with the larval stages of *A. galli* as recorded by Ackert (1) they approximated the second- and third-stage larvae. The third-stage larvae were 6.3 mm. long; the average length of this stage in *A. galli* is stated by Ackert to be 6.4 mm. Other characters were similar to those described by Ackert. Three distinct lips are present, showing papillae (Fig. 17). In the male there is a distinct pre-anal sucker as well as certain caudal papillae (Fig. 16), and several cells comprising the primordium of the spicules can be seen. The second-stage larvae are 3.8 mm. long; Ackert figures *A. galli* second-stage larvae as 4.2 mm. long. They have lips just beginning to form, an anal prominence, but no pre-anal sucker.

Of the pigeons kept at the Institute, eight were autopsied; all but one harbored capillarids and all but three, ascaridias. The incidence of the infection is given in Table I. Fecal examinations showed that the 11 Tumblers as well as a third of the Homers were infected with ascaridias. It must be remembered, however, that in some cases a pigeon may harbor a single male and consequently not show eggs in the faeces. In the case of capillarids, fecal examinations are not as satisfactory, as the eggs appear sporadically. However, at least three quarters of the birds were infected with capillarids..

TABLE I

PIGEON PARASITES FOUND

Date	No. of pigeons	Parasites
Nov. 26	66	16 echinostomes 2 larval nematodes
Dec. 6	8	Negative
Jan. 18	78	1 echinostome 10 larval ascaridias 3 capillarids
Mar. 2	1	3 capillarids 1 ascaridia
Mar. 2	1	1 capillarid 10 ascaridias
Mar. 3	1	1 capillarid 2 ascaridias
Mar. 3	1	Negative
Mar. 26	1	1 ascaridia 7 capillarids
Mar. 28	1	3 capillarids
Apr. 17	1	7 ascaridias 1 capillarid

The ascaridias were identified as *A. columbae* and the capillarids as *C. columbae*.

The fact that from the Gatehouse material 12 larval ascaridias were obtained, and no adults, suggests that probably a definite age resistance to *A. columbae* is shown by the pigeon, as is shown to *A. galli* by the fowl (Ackert *et al.* (3)).

The pigeons exhibit some evidence of a strain resistance to *A. columbae*. This is suggested by the higher proportion of infected birds among Tumblers than among the Homers, and the fact that

one of the Tumbler pigeons yielded a larva about 4.0 mm. in length, whereas in the Homers experimental infection did not produce a larva over 48 hours (about 0.36 mm.).

Description of Parasites

Ascaridia Dujardin, 1845

Generic diagnosis (From York and Maplestone). Generally with cuticular lateral flanges: oesophagus club-shaped, but without a posterior bulb. Male: pre-anal sucker slightly prominent with a chitinous rim; caudal alae narrow; papillae relatively large; spicules equal or subequal; gubernaculum absent. Female: vulva near the middle of the body; uteri opposed. Oviparous eggs with a thick shell.

A. columbae Gmelin, 1789

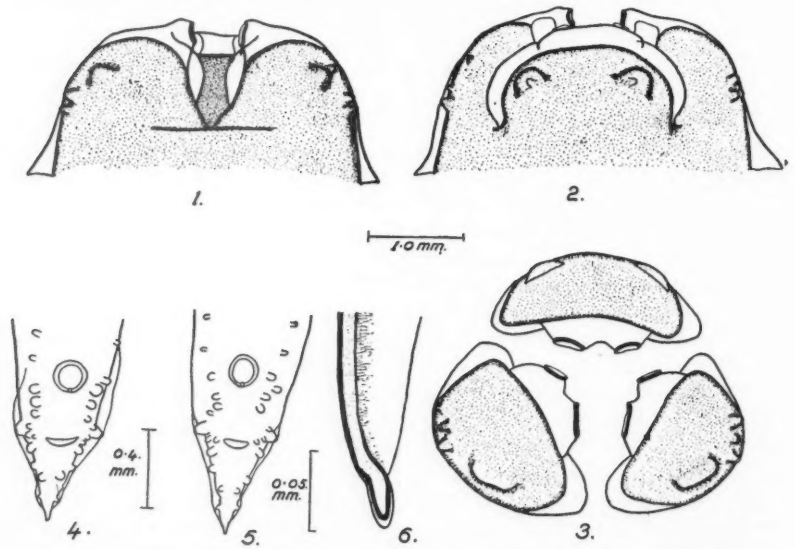
Specific description. There is a great variation in the length of *A. columbae* as given by different authors. The length ranges from 16–25 mm. for the males and 20–35 mm. for the females, as stated by Neumann and MacQueen (23), to 60–70 mm. for the males and 70–90 mm. for the females, as stated by Baylis and Daubney (6). The lengths noted by other authors fall between these two extremes. It should be noted that this parasite occurs in a variety of hosts, and Cram (7) suggests that the variation in length may be associated with this fact. In my specimens the males range from 35 to 42 mm., the females from 43 to 54 mm.

At the anterior extremity are two semi-elliptical membranes, projections of the cuticle. They are about the same length as the pharynx and are marked for part of their width with continuations of the striations of the body cuticle. The pharynx is 2.5 to 2.62 mm. long.

The number of caudal papillae in the male has been given by different authors as 10 to 14 pairs. Johnston (15) figures 13 on one side and 14 on the other. Irwin Smith (14) figures 13 pairs, Yamaguti (31) 13 pairs, and Baylis and Daubney (6) 14 pairs. In the material studied 14 pairs seems to be the normal number, but it is not unusual to find papillae missing or duplicated. The normal arrangement appears to be five pairs of post-anal papillae, of which the third pair is small and inconspicuous and in certain individuals appears to be lacking, a condition which Baylis and Daubney also noted. There is an adanal group of four pairs, one large lateral and three smaller, more ventral pairs. No changes in the number of these papillae were noted, but there are slight variations in different individuals in the relative positions of the three more ventral pairs. Between the anus and the genital sucker are three pairs of rather large papillae. It is not uncommon to find an extra papilla, that is, four on one side and three on the other, and in one specimen there are only two on one side and three on the other. Near the level of the anterior margin of the sucker is a pair more ventrally placed, and anterior to that is found the final pair. The last two pairs mentioned are quite small and inconspicuous. In one of the specimens examined the last pair appeared to be missing; according to Baylis and Daubney, it may be duplicated.

In the male, the anus is 0.44 to 0.48 mm. and the posterior margin of the genital sucker 0.58 to 0.72 mm. from the tip of the tail. The genital sucker varies in outline from circular to sub-circular and sizes noted were 176 × 176μ, 184 × 175μ and 176 × 169μ. The spicules varied from 1.05 to 1.72 mm. in length and were 57μ wide. The proximal part is slightly expanded and the distal extremity tapers abruptly to a blunt point; the posterior part has a cuticular expansion on the inner surface (Fig. 6).

In the female the anus is 0.9 to 1.1 mm. from the tip of the tail.



FIGS. 1–6. *Ascaridia columbae.* FIG. 1. *Head, ventral view.* FIG. 2. *Head, dorsal view.* FIG. 3. *Head, front view.* FIGS. 4 AND 5. *Caudal papillae of male.* FIG. 6. *Tip of spicule.*

Although the lip structures are of considerable taxonomic importance they have been accorded little attention in *A. columbae.* The lips are similar to those of *A. galli,* as described by Ackert (1), that is, three prominent lips, one dorsal and two subventral, the distal margins of each being divided into three lobes, one medium and two lateral. However, where in *A. galli* there is a dentigerous ridge on the inner surface of each medium lobe, in *A. columbae* the inner surface has two saucer-shaped sucker-like structures, placed in such a position that there are three pairs, the members of each pair directly opposing one another (Figs. 2 and 3). They probably function as a hold-fast organ. In reference to the labial papillae, Johnston (15) mentions two small papillae on the dorsal lip. Yamaguti (31) states that, "the three lips are 0.07 to 0.11 mm. long and bear each two papillae, those of the dorsal lip being smaller and inconspicuous." A careful study of the labial papillae showed that there

are two large papillae on the dorsal lip (Fig. 2); on each of the ventral lips there is one large, ventrally placed papilla, and a group of three smaller ones more dorsally placed (Fig. 1); in one specimen the dorsal group was found to consist of four papillae. This suggests that the number of this group may vary, as in the case of the caudal papillae of the male.

Very characteristic of *A. columbae* is the presence of large vesicular-like structures that fill the interior of the body of both sexes. Dujardin and Irwin Smith who appear to be the only authors to mention them, refer to them as "orbicular corpuscles" and consider them to be present only in the female.

Baylis and Daubney (6) mention 26 to 30 pairs of cervical papillae. I have been unable to recognize them.

The eggs vary in size from 78 to 84μ long by 49μ wide.

Capillaria Zeder, 1800

Generic diagnosis (From York and Maplestone). Body capillary; mouth simple; cuticule with bacillary bands dorsal, ventral or lateral in position; oesophagus long and gradually increasing in size posteriorly. Male: anus terminal or sub-terminal, small membranous caudal alae or bursa-like structure present or absent; spicule long and slender, with or without spines on its surface. Female: vulva near termination of oesophagus. Oviparous eggs lemon shaped, with the usual opercular plugs at the poles.

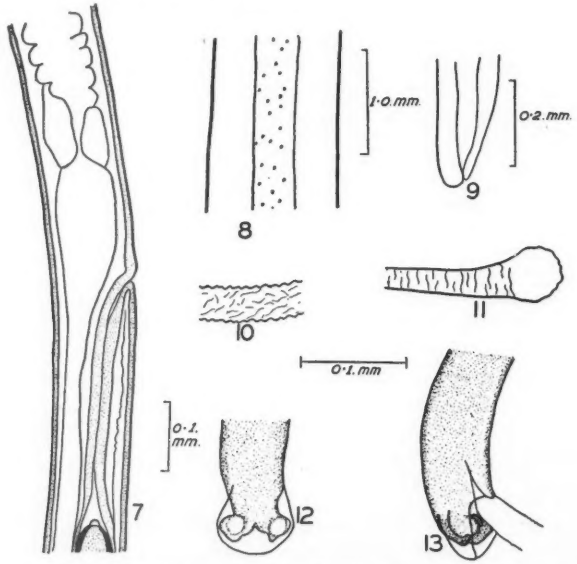
C. columbae Rudolphi, 1819

Specific description. Long thread-like worms with a white translucent appearance; cuticle finely striated transversely; two broad lateral bacillary bands which thin out at both ends, the surface of the bands being irregularly dotted with black spots; cells of the oesophagus rectangular, annulated, with a prominent nucleus in the centre; at the region where the oesophagus and the intestine meet are two smaller non-annulated cells on each side of the digestive tube (Fig. 7).

Male: Only one entire male was recovered. It measures 11.4 mm. in length. According to other authors the length may vary from 8.1 to 11.7 mm. The oesophagus is slightly less than half the entire length or 5.53 mm. The cloacal aperture is nearly terminal and is surrounded by a membranous bursa-like structure. Supporting this membrane are two broad rays, each of which appears to have a secondary outgrowth (Figs. 12 and 13). The spicule in two specimens measures 1.56 mm. and 1.4 mm.; it has a blunt, rounded point and at the proximal extremity expands like a trumpet (Fig. 11). In the entire male specimen the spicule sheath is extruded and measures 2.94 mm. It is irregularly annulated and covered with fine markings (Fig. 10). At the widest part it measures 0.034 mm.

Female: Three females measured 14 mm., 15.1 mm. and 19 mm. This is greater than the lengths recorded by other authors. Graybill (13) mentions 10 to 12.3 mm., Irwin Smith (14), 13 to 16.24 mm. and Morgan (21) 10.1 to 14.5 mm. as the lengths for the females of *C. columbae* they examined. In two females the oesophagus is slightly more than one third of the total length, or 39%. The posterior extremity tapers to a blunt point with the cloacal

aperture slightly sub-terminal (Fig. 9). The vulva is situated near the junction of the oesophagus and the intestine, and has slightly protruding lips (Fig. 7). The vagina passes backwards.



FIGS. 7–13. *Capillaria columbae.* FIG. 7. *Female, in region of vulva; lateral view.* FIG. 8. *Lateral view showing bacillary band.* FIG. 9. *Posterior extremity of female.* FIG. 10. *Anterior end of spicule.* FIG. 11. *Spicule sheath.* FIG. 12. *Posterior extremity of male, ventral view.* FIG. 13. *Posterior extremity of male, lateral view.*

The eggs are ellipsoid, brownish in color but often appear greenish when collected in the feces. They have a thick shell discontinued at either pole where two opercular plugs are present. Egg sizes vary from 47 to 72μ long and 24 to 31μ wide.

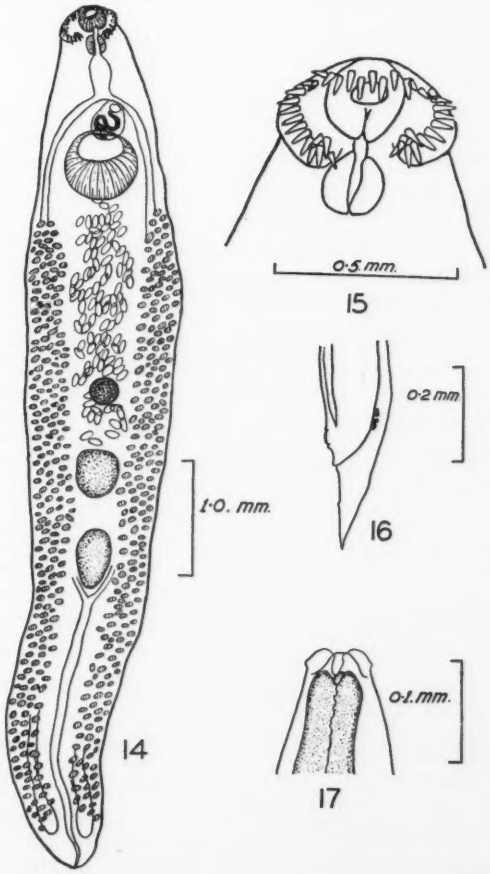
Echinostoma paraulum (Dietz, 1909)

This appears to be the first time *E. paraulum* has been reported in North America.

In 1925 Zunker (35) described this parasite from the pigeon as new under the name *Echinostoma columbae.* Later Mönnig (20) states it to be a synonym of *Echinopharyphium paraulum* Dietz, 1909. Unfortunately I have been unable to see the original description by Dietz, but the characteristics revealed by my specimens, although resembling Zunker's description, would not place them in the genus *Echinoparyphium.* According to Dietz (10) ". . . . im Gegensatz zu allen andern von mir unterschiedenen Echinostomidengattungen sind jedoch die Randstacheln beider Reihen nicht gleich gross, sondern die Stächeln der oralen Reihe kleiner als die der aboralen." While it is true that on the average the aboral spines are longer than the oral spines the difference

is very slight, and as there is an overlapping of sizes, I believe that the trematode should be kept in the genus *Echinostoma*. Therefore, as I consider *Echinostoma columbae* to be a synonym of the echinostome described by Dietz, the name should be changed to *Echinostoma paraulum*.

Specific description. Elongate worm measuring from 4.7 to 8.5 mm. in length. In my specimens the greatest width occurs immediately posterior to the acetabulum and varies from 1.06 to 1.3 mm. In Zunker's figure the greatest width is in the posterior part of the body. The head crown bears two ventral groups of five spines, the arrangement of which is not constant,



FIGS. 14–15. *Echinostoma paraulum.* FIG. 14. *Ventral view.* FIG. 15. *Ventral view of anterior end, showing head crown.*

FIGS. 16–17. *Ascaridia columbae.* FIG. 16. *Posterior extremity of third stage male larva.* FIG. 17. *Anterior extremity of third stage larva.*

and 27 marginal spines (Fig. 15). On the dorsal surface the spines tend to form two rows, and the length of the aboral spines varies from 68 to 80μ while those of the oral are from 66 to 70μ. No spines are found on the body, but, as has been suggested by Mönnig (20) in reference to Zunker's specimens, they may have been lost during post mortem changes. The oral sucker is subcircular and is 0.17 to 0.22 mm. in diameter, the ventral sucker 0.52 to 0.64 mm. and the pharynx 0.17 to 0.22 mm. The oesophagus is 0.36 mm. long and bifurcates halfway between the pharynx and acetabulum. The two digestive caeca reach to near the posterior tip of the body.

The testes are usually oval to circular in outline but may be lobed, tandem, and lie in the third quarter of the body. Zunker (35) states that in the mature specimens he examined, the testes were constricted in the middle. The cirrus pouch is found between the point of bifurcation of the oesophagus and the acetabulum, usually partly overlapping the latter. The ovary is subcircular, located just anterior to the anterior testes. The uterus is a long convoluted tube. The vitellaria are extensive and reach from the posterior border of the acetabulum to near the posterior end of the body. The eggs are oval, and are 96 to 98μ long by 56 to 64μ wide.

The excretory system is Y-shaped with a long narrow base bifurcating immediately caudad of the posterior testis, the two branches running forward to the posterior border of the acetabulum.

In general, the characters as described above agree quite consistently with those stated by Zunker. The differences in shape of body and outline of the testis are not constant characters and therefore could not form the basis for the description of a new species.

Check List of Parasites of Pigeons

Parasite	Distribution
Trematoda	
STRIGEIDAE	
Apatemon gracilis Rudolphi, 1819.	?
HARMOSTOMIDAE	
Brachylaemus commutatus Diesing, 1855.	South Europe, North Africa, England, Indo-China.
B. mazzantii Travassos, 1917.	Brazil.
ECHINOSTOMIDAE	
Hypodaraeum conoideum Bloch, 1782.	Europe.
Echinoparyphium recurvatum Linstow, 1873.	Europe.
Echinostoma paraulum Dietz, 1909.	Europe, Java, Canada.
E. robustum Yamaguti, 1935.	Japan.

Check List of Parasites of Pigeons—*Concluded*

Parasite	Distribution
Cestoda	
BOTHRIOCEPHALIDAE	
Diphyllobothrium erinacei (Rudolphi, 1819), 2nd stage larvae.	?
ANOPLOCEPHALIDAE	
Aporina delafondi Railliet, 1892.	Asia, Africa, Europe, South America, North America.
DILEPIDIDAE	
Choanotaenia infundibulum Bloch, 1779. ?.	World-wide.
HYMENOLEPIDAE	
Hymenolepis (Hymenolepis) serrata Fuhrmann, 1906.	Europe.
H. (Hymenolepis) serrata var. *birmaniea* Meggitt, 1924.	India.
H. (Weinlandia) columbae Zeder, 1800.	Europe, India, North America.
DAVAINIIDAE	
Cotugnia cuneata var. *tenuis* Meggitt, 1924.	Burma, India, Egypt.
C. cuneata var. *nervosa* Meggitt, 1924.	Burma, India, Japan.
Raillietina (R.) micracantha Fuhrmann, 1909.	Africa, Europe, Asia.
R. (R.) nagpurensis Moghe, 1925.	India.
R. (R.) torquata Meggitt, 1924.	Burma.
Raillietina Skrjabinia bonini (columbae) (Megnin, 1899).	England, Europe.
R. (R.) clerci Fuhrmann, 1920.	Asia, Africa.
R. (R.) joyeuxi Lopez Neyra, 1929.	Spain.
R. (R.) tunetensis Joyeux & Houdemer, 1928.	Tunis, India.
R. (R.) taiwanensis Yamaguti, 1935.	Japan.
R. (R.) echinobothridia Megnin, 1881.	Cosmopolitan.
R. (R.) volzi Fuhrmann, 1905.	Asia, Sumatra.
R. Fuhrmanetta crassula Rudolphi, 1819.	Europe, Africa, North America, South America.
Nematoda	
HETERAKIDAE	
Ascaridia columbae Gmelin, 1789.	North America, South America, Europe, Asia, Africa, Australia.
SPIRURIDAE	
Acuaria (Dispharynx) spiralis Molin, 1858.	North America, South America, Europe, Asia, Africa.
Tetrameres (Tetrameres) fissispina (Diesing, 1861) Travassos, 1914.	North America, Europe, Asia, Africa.
T. (T.) confusa Travassos, 1919.	South America.
TRICHINELLIDAE	
Capillaria columbae Rudolphi, 1819.	North America, South America, Asia, Africa, Australia.
FILARIIDAE	
Eulimdana clava Wedl, 1855.	Europe.
E. mazzantii Railliet, 1895.	Europe.
STRONGYLIDAE	
Syngamus trachealis Siebold, 1836 ?	Europe.
TRICHOSTRONGYLIDAE	
Ornithostrongylus quadriradiatus Stevenson, 1904.	North America, Australia, Cuba, Natal.

The following helminths have been experimentally introduced into the domestic pigeon.

TREMATODES:— *Brachylaemus fuscatus, Echinostoma erraticum, E. exile, E. parvum.*

NEMATODES:—*Oxyspirura parvovum, Tetrameres (Microtetrameres) helix, Trichinella spiralis.*

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